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# ANNALS
OF
# TROPICAL MEDICINE AND PARASITOLOGY

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VOLUME XVIII
(April 30, 1924, to December 30, 1924)

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# A MALARIA PARASITE OF THE CHIMPANZEE

BY

B. BLACKLOCK

AND

S. ADLER

*From the Sir A. L. Jones Research Laboratory, Freetown*

(*Received for publication* 10 *December*, 1923)

Reichenow (1920) described in chimpanzees in the Cameroons malaria parasites, indistinguishable from the three species which occur in human beings, and suggested that the chimpanzee is naturally affected with human malaria. Mesnil and Roubaud's successful inoculation of *Plasmodium vivax* into a chimpanzee supports Reichenow's conclusion as far as *P. vivax* is concerned.

Mesnil and Roubaud failed to infect a chimpanzee with *Plasmodium falciparum*, either by direct inoculation, or by infective mosquitoes. This experiment, however, cannot be taken as conclusive, as Reichenow and Adler have produced evidence to show that chimpanzees acquire immunity to malaria, after attacks in early life. A conclusive experiment could only be carried out on a very young animal which had not yet been attacked with malaria.

We were able to carry out such an experiment on October 30, 1922. A young male chimpanzee three months old was under observation since September 11, 1922, and its blood on repeated examination was negative; 3 c.c. of human blood heavily infected with *Plasmodium falciparum* were citrated and injected subcutaneously into the animal. The injection was given two and one-half hours after the blood was withdrawn from the human case.

The animal did not become infected with *P. falciparum*, and this, in conjunction with Blacklock and Adler's (1922) failure to infect two Europeans with infected chimpanzee's blood is evidence

that *Plasmodium falciparum* is a different species from the similar parasite which occurs in chimpanzee's blood. We, therefore, propose the name of *Plasmodium reichenowi* for the latter, in consideration of the fact that this observer first found the parasite in chimpanzees.

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# ON THE ANATOMY OF *TAENIA FESTIVA*, RUDOLPHI, 1819

BY

G. THEILER.

*From the Parasitology Department of the Liverpool School of Tropical Medicine*

(*Received for publication* 11 *December*, 1923)

*CITTOTAENIA FESTIVA* (Rudolphi, 1819).

Synonym:—*Moniezia festiva*, Blanchard, 1891.

In 1819 Rudolphi described a cestode, *Taenia festiva*, from the bile duct and gall bladder of *Halmaturi gigantei* (= *Macropus giganteus*). This was later figured by Bremser in 1824, and was identified by Cobbold (1879) as occurring in *Macropus derbyanus*. Blanchard, in 1891, included it in his genus *Moniezia*. As Rudolphi's description does not give sufficient anatomical details, it has remained in Cestode Classification as a doubtful *Moniezia*.

Since 1819 other cestodes with bilateral pores have been described from kangaroos and allied animals, viz.:—

*Taenia fimbriata*, Krefft, 1873 = *Taenia? krefftii*, Johnston, 1909.

*Taenia bipapillosa*, Leidy, 1875, from a wombat.

*Cittotaenia zschokkei*, Janicki, 1905, from *Macropus* sp.?

*Moniezia diaphana*, Zschokke, 1907, from **Phaseolomys wombat*.

*Cittotaenia bancrofti*, Johnston, 1912, from *Onychogale frenata*.

*Cittotaenia lagorchestis*, Lewis, 1914, from *Lagorchestes conspicillatus*.

*Cittotaenia villosa*, Lewis, 1914, from *Lagorchestes conspicillatus*.†

---

* Johnston (1911) questions the correct identification of this host or the locality from which the host is recorded, as *P. wombat* does not occur on the mainland but in Tasmania or the neighbouring island of the Bass Straits.

† *T. phalangistae*, Krefft (1873), from the common opossum need not any longer be taken into account, as it is not recognisable from the description.

Johnston (1909) stated that he had recently examined superficially some material from *Macropus derbyanus* which apparently consisted of *Moniezia festiva*. He also mentioned that he has in his possession a number of tapeworms agreeing externally with *Taenia bipapillosa*, taken from a wombat, *Phaseolomys mitchelli*, and remarked ' I hope that before long I shall be able to make known the structure of these two species of *Moniezia*.' In 1912 he described a *Cittotaenia*, *C. bancrofti*, from a wallaby, *Onychogale frenata*, but so far, neither in this nor any other publication, has he made further mention of his specimen of *M. bipapillosa*.

All the double-pored cestodes from *Monotremes* and Marsupials which have been carefully examined have proved to belong to some species of the genus *Cittotaenia*, e.g., *C. zschokkei*, *C. bancrofti*, *C. diaphana*, *C. lagorchestis*.

According to the data given of *T. fimbriata*, Krefft (= *T.? krefftii*, Johnston), we gather that we are dealing either with a *Moniezia* or a *Cittotaenia*, but further identification is impossible.

Leidy's description of *T. bipapillosa* is imperfect, but Johnston points out its similarity with the figures of *Taenia festiva* as given by Bremser. We may, therefore, either assume that *T. bipapillosa* is synonymous with *T. festiva* or, owing to lack of anatomical data, leave it altogether out of account.

Examination of Rudolphi's original description and of Bremser's drawings, reveals the fact that we may have ' an appearance as of four ovaries (uteri),' and that the ovaries (uteri) do not meet in the median field. At no stage in the development of a *Moniezia* is this appearance as of four uteri to be seen, nor do the uteri ever have a clear space in the median line. In a few species (e.g., *C. variabilis* and *C. diaphana*) of the genus *Cittotaenia*, however, the two uteri arise separately and may not meet in the median field. Under the circumstances we can assume that Rudolphi's *Taenia festiva* is a *Cittotaenia* and not a *Moniezia*.

This assumption is borne out by the study of cestode material collected by Dr. Bruck from a kangaroo in Golden Gate Park, California. Mr. Southwell, of the Liverpool School of Tropical Medicine, to whom it had been sent for identification, most kindly handed it over to me. The material, consisting of one complete worm and several fragments, presented upon external and superficial

examination all the characteristics of *Taenia festiva*, as described by Rudolphi, but upon detailed investigation proved to be a typical *Cittotaenia*, with the following characters:—

*CITTOTAENIA FESTIVA* (Rudolphi, 1819)

The worm is about 13 cm. long ('8 to 10 pollices,' Rudolphi) and up to 5 mm. broad ('2 to 3 lineas,' Rudolphi); fairly thick in the contracted portions, thin in some of the relaxed fragments ('thin and transparent,' Rudolphi). Pores bilateral, opening in the posterior portion of the lateral margins.

The segments vary in shape according to the state of contraction of the worm, and are broader than long. Mature segments average 2·6 mm. broad by 0·35 mm. long; gravid segments are 5 mm. broad by 0·72 mm. long. In the mature portions of the worm, the posterior border of each segment overlaps at least one-half of the next segment.

*Head* (fig. 1). This is a short truncate cone, 0·54 mm. in length, with its greatest diameter 0·68 mm. near the anterior end. A marked

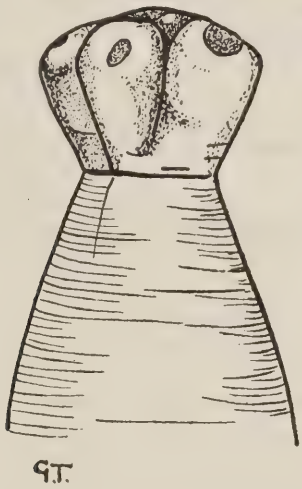

FIG. 1. *Cittotaenia festiva.* Head.

lobing is present, so that each sucker is prominent (as figured by Bremser). A certain amount of contraction is apparent. The openings of the suckers are circular and face anteriorly. Viewed *en face*, the outline of the head is square, four lobed.

*Neck.* A deep constriction separates the head from the rest of the strobila. It is difficult to say whether a neck is present or not, as this portion of the specimen is very contracted.

*Nervous system.* Ventral to the genital canals.

*Muscular system.* Not very strongly developed; the longitudinal muscles do not show any arrangement in definite rows.

*Excretory system* (fig. 3). Ventral to the genital canals. The dorsal vessel lies dorso-lateral to the ventral vessel. In some segments there appear to be transverse canals to the dorsal vessels.

*Male genitalia* (fig. 2). *Testes.* The testes are arranged in two groups. They number on an average forty-eight to fifty-five on the left side and sixty-four to sixty-eight on the right side. They do

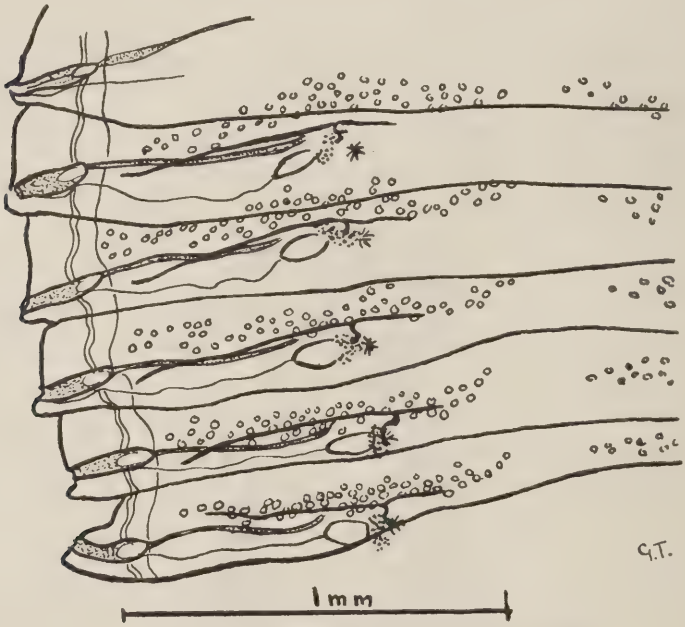


Fig. 2. *C. festiva. In toto* preparation of mature segment.

not meet in the median field, while laterally they reach to the longitudinal vessels. They are anterior and dorsal to the female genitalia.

*Vas deferens* (fig. 2). In the young segments the vas deferens appears as a straight narrow tube. Later, however, it enlarges into an elongate swelling before its entrance into the cirrus pouch; within the cirrus pouch it forms a seminal vesicle. Just median of the

enlargement, it describes a few coils and then continues as a straight and narrow tube (fig. 4). The cirrus pouch is large, slightly elongate and, before the seminal vesicle is filled with sperms, measures 240$\mu$ to 280$\mu$ by 44$\mu$ to 52$\mu$. It reaches beyond the longitudinal canals, which it crosses dorsally.

*Female genitalia* (fig. 3). The fairly large mass of the shell gland adjoins the receptaculum seminis; median to this is the ovary and posteriorly the vitelline glands. The ovary may underlie the shell gland ventrally, the main bulk of the ovary lies ventral, and the vitelline gland is dorsal in position. The shell gland lies at a level between these two.

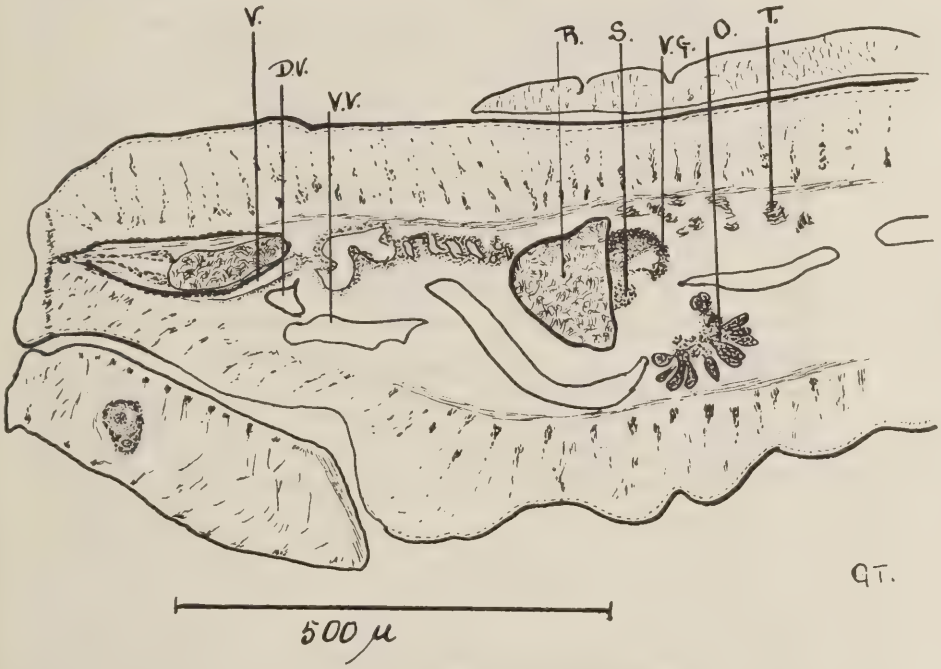


Fig. 3. *C. festiva.* Section of mature segment.

*V.*—Vagina; *D.V.*—Dorsal longitudinal vessel; *V.V.*—Ventral longitudinal vessel; *R.*—Receptaculum seminis; *S.*—Shell gland; *V.G.*—Vitellogene gland; *O.*—Ovary; *T.*—Testes.

*Receptaculum seminis and vagina.* The vagina is ventral and posterior to the cirrus pouch on both sides. It opens on the edge of the slight collar surrounding the male aperture. In older segments the vagina disappears. The receptaculum seminis may attain enormous proportions; when filled with sperms it extends from the anterior to the posterior margin of the segment (fig. 4).

In older proglottides the two receptacula are pushed posteriorly until they divide the posterior margin of the segment into three equal parts (fig. 5).

*Oviduct.* After receiving the vitelline duct the large oviduct runs anteriorly for a short distance, then dips and runs diagonally, ventrally and anteriorly, meeting the uterus at a right angle.

*Uterus* (figs. 2 and 4). This is at first a narrow tube (one to each set of genitalia), which eventually extends from the median line to the longitudinal vessels. It may cross these latter dorsally.

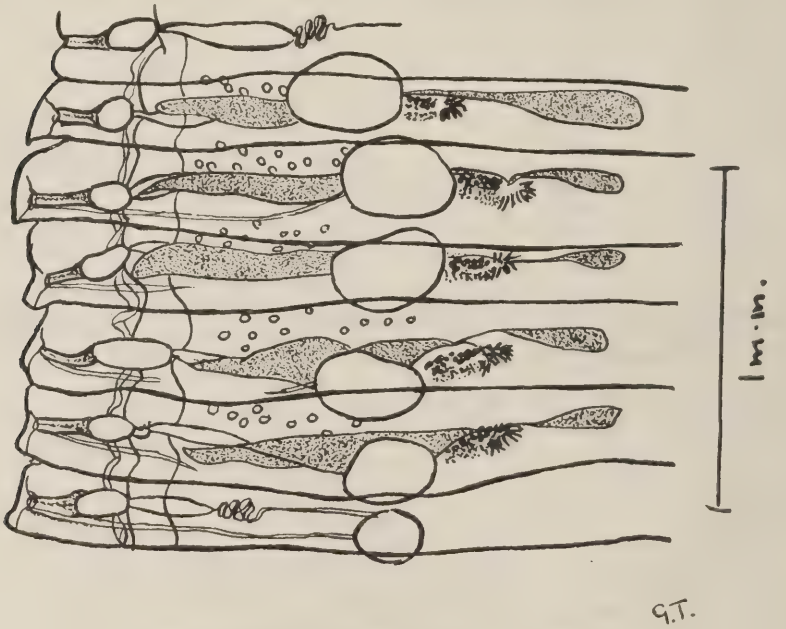


Fig. 4. *C. festiva.* Showing development of uterus.

The two uteri may meet in the median line, but they never unite. As the eggs develop the two branches of the uterus merely expand into two large pouches, giving in some segments the appearance of four separate units (as described by Rudolphi) until eventually they practically fill the whole segment (figs. 5 and 6).

*Eggs.* The eggs were not quite mature. Those examined in carbolic acid measured 46$\mu$ to 50$\mu$ in diameter, the bulb of the pyriform body 20$\mu$ to 23$\mu$. The horns are long, coiled and tapering.

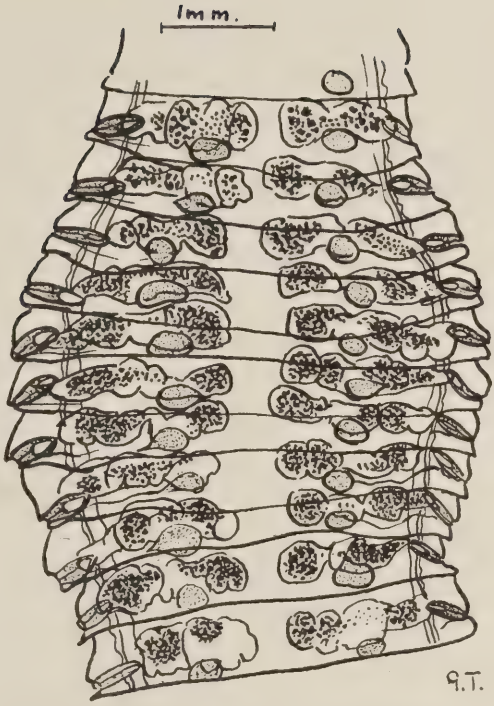


FIG. 5. *C. festiva.* Showing development of uterus.

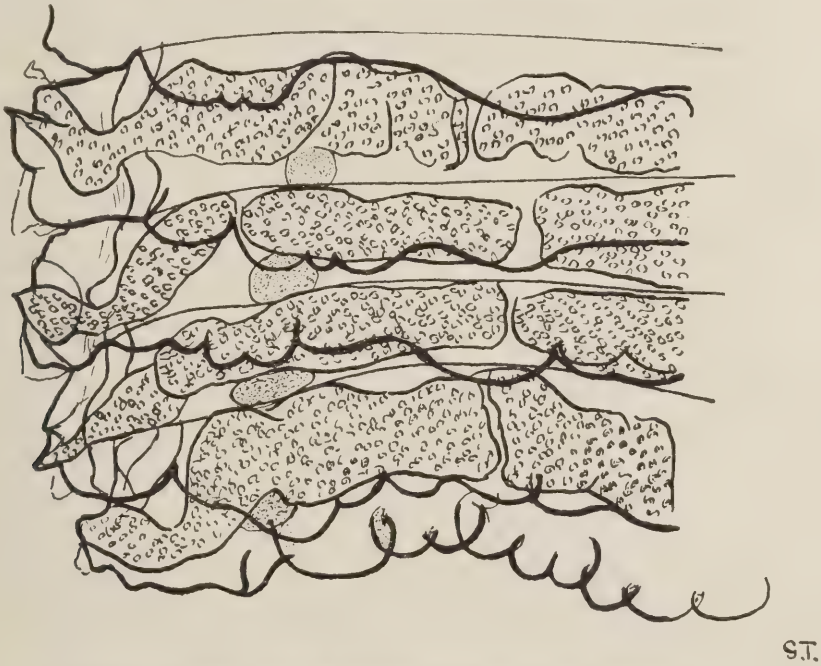


FIG. 6. *C. festiva.* Showing development of uterus; much contracted.

## DISCUSSION

The *Cittotaenia* occurring in Marsupials may be divided into two classes :—

(1) Fimbriated :

*C. zschokkei*,
*C. lagorchestis*,
*C. villosa*.

(2) Non-fimbriated :

*C. diaphana*,
*C. bancrofti*,
*C. festiva*.

Krefft (1873) describes a fimbriated cestode, *T. fimbriata*, from a *Haematurus* spp., but as his description is incomplete, his species can now hardly stand.

The following table gives a comparison of the main characters of the three non-fimbriated species :—

TABLE I.

| | *C. diaphana* | *C. bancrofti* | *C. festiva* |
|---|---|---|---|
| Length ... ... | 60 to 90 mm. | 150 mm. | 130 mm. |
| Maximum breadth | 3·5 to 3·9 mm. | 14 mm. (?) | 5 mm. |
| Diameter of Scolex | 1 mm. | 1·9 mm. | 0·68 mm. |
| Cirrus pouch ... | Slender and elongate | Large, elongate, 0·8 to 1 mm. | Elongate. |
| Cirrus ... ... | Short, thread-like | With spine | Thread-like |
| Vas deferens ... | Much convoluted | Closely coiled near cirrus sac | Straight course, except for a few coils behind the enlargement. |
| Testes ... ... | Two separate groups | Not distinguishable in the specimens | Two separate groups. |
| Ovaries ... ... | Dorsal, median, anterior | Median and anterior | Ventral, median, anterior. |
| Vitellogene glands | Ventral, posterior | Posterior | Dorsal, posterior. |
| Uterus ... ... | Two. May pass between excretory vessels | Two | Two. Cross excretory vessels dorsally only. |
| Eggs ... ... | Horns short | — | Horns long, tapering coiled. |

*C. festiva* is thus seen to approach *C. diaphana* most closely, the main points of difference being that in *C. diaphana* the vas deferens is much convoluted (fig. 7), and the ovaries occupy a dorsal position, whereas in *C. festiva* there are hardly any convolutions in the vas deferens, and the ovaries lie ventral. For *C. diaphana* the horns of the pyriform body are given as short, whereas in my specimen, which was not quite mature, the horns were long and tapering. *C. diaphana* and *C. festiva* so closely resemble one another in all

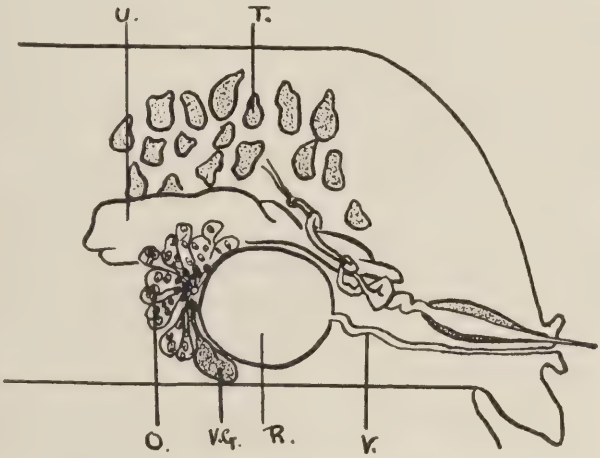


FIG. 7. *C. diaphana.* Mature proglottis after Zschokke.

*U.*—Uterus; *T.*—Testes; *O.*—Ovary; *V.G.*—Vitellogene gland; *R.*—Receptaculum seminis; *V.*—Vagina.

respects (except for a few slight differences which might easily be due to contraction) that the writer is inclined to believe that they are identical. The dorsal position attributed to the ovary by Zschokke, however, cannot be explained away by contraction; so until more work has been done on the *Cittotaenia* of Marsupials, *C. diaphana* and *C. festiva* must be considered as two separate species.

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Since going to press, the writer's attention has been drawn to a publication by Nybelin (1917). In this paper, upon the examination of Rudolphi's original specimen and the new material

at his disposal, Nybelin decides to create a new genus—*Hepatotaenia* for *T. festiva*, Rud. 1819. The genus *Hepatotaenia* differs from the genus *Cittotaenia* in the following respects:—Testes arranged in two groups which do not meet in the median field, and are near to the anterior end of the female genitalia. Vagina opens behind the male genital opening. The two sacciform uteri never fuse with each other.

In this genus he includes:—

*H. festiva*, Rudolphi.
*H. diaphana*, Zschokke.
*H. fellicola*, Nybelin (1917).

The writer's specimen agrees in every respect with Nybelin's description of Rudolphi's *T. festiva*, and is, therefore, to be considered as a *Hepatotaenia* and not a *Cittotaenia*.

For the species *C. bancrofti*, *C. zschokkei* and *C. lagorchestis*, Nybelin creates the genus *Progamotaenia*, which differs from *Hepatotaenia* in that the gravid segments contain but one sacciform uterus, which may or may not have been double when young.

The eggs possess no pyriform apparatus.

Nybelin is inclined to put *C. papillosa* in a genus by itself, as in this species the testes are arranged in two large testicular sacs.

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# TWO RARE SPECIMENS OF HUMAN CESTODES

BY

PROFESSOR SADAO YOSHIDA, D.Sc.

AND

SEIJI OGATA, M.D.

*Pathological Department, Osaka Medical College, Japan*

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PLATE I

In May, 1923, the writers received two rare specimens of human cestodes from their colleague, Dr. I. Maeda, to whom they had been presented by Mr. Nakasuga, a practitioner in Himeji city, near Osaka. One of the specimens has been identified as *Dibothriocephalus parvus*, Stephens, 1907. Dr. Leon (1915) briefly announced that he had found a case in Roumania, but hitherto the worm has never been reported in Japan. The other specimen is a remarkable malformation of *D. latus*.

## 1. *DIBOTHRIOCEPHALUS PARVUS*, Stephens, 1907

The present specimen was obtained in August, 1922, from a boy aged nine years, the son of a restaurant proprietor in Himeji city. The patient had been in the habit of eating fish from various parts of the country. He had never left his native home except on a trip to Osaka, in the spring of last year.

The strobila without the head measures 2·315 metres in length, and consists of about 1,150 proglottides. During the nine months that had elapsed since the expulsion of the worm, the patient had not shown any sign of the presence of a parasite either in the faeces or in the way of symptoms. Hence it is probable that the head was passed with the strobila, but was overlooked.

The anterior segment is 0·55 mm. broad and 1·0 mm. long, the posterior segment 5·5 mm. broad and 3·0 mm. long, the maximum

segment being 7 mm. broad and 3 mm. long, and occurring six to ten centimetres from the posterior end. The segments of the strobila gradually increase in breadth and in length posteriorly; the former dimension is always greater than the latter, except in a few segments at the anterior extremity which are longer than broad, probably because of the elongated state of the worm. This regular increase in the dimensions of the segments towards the posterior end is interrupted here and there by the irregular extensions and contractions of the segments. The segments, 100 cm. behind the anterior end, are approximately quadrate in shape, both the length and breadth being 3·5 to 4·0 mm. Actual measurements of some segments at various distances from the anterior extremity are as follows:—

| | Breadth | Length | | Breadth | Length |
|---|---|---|---|---|---|
| 20th Segment ... | 1·5 mm. | 1·0 mm. | 205th Segment ... | 2·6 mm. | 0·6 mm. |
| 500th Segment ... | 3·5 mm. | 2·0 mm. | 623rd Segment ... | 4·0 mm. | 3·5 mm. |
| 1000th Segment ... | 6·0 mm. | 3·0 mm. | 1030th Segment ... | 7·0 mm. | 3·5 mm. |

(The number of the segments given above does not indicate the first segment of the corresponding breadth and length; for instance, the 20th segment is not necessarily the first one of the segments which are 1·5 mm. broad and 1·0 mm. long.)

The posterior border of each segment overlaps the anterior border of the segment following it, and consequently, the lateral margins of the worm present a serrated appearance which differs a little from the description given by Stephens. The surface of the worm is much wrinkled with transverse and longitudinal furrows. The state of corrugation varies according to the condition of the contraction of the segments; thus, in the most extended segments, the transverse wrinkles disappear although the longitudinal ones still exist, while in the contracted segments the transverse and longitudinal furrows are numerous and most conspicuous. Throughout the entire strobila, two (four in all) distinct longitudinal furrows run almost uninterruptedly along the submedian lines on both ventral and dorsal surfaces. Probably these continuous longitudinal furrows are situated on the lines corresponding to the

lateral nerve cords; they lie at a distance of 2 mm. from the lateral margins of a segment 7 mm. wide.

The cirrus opening is situated about one-third to one-fourth of the length of the segment from the anterior margin; the uterine openings cannot be easily made out with a hand-lens owing to the distortion caused by wrinkles on the surface.

The yellowish mass of the uterine loops can be recognised by the naked eye 50 cm. from the anterior end of the worm. As the uterine tube enlarges, the loops form yellowish globular tubercles upon the dorsal surface. Under the microscope, the uterus appears as a central rosette with four to six loops on each side.

The longitudinal layer of the parenchymal musculature is diffuse and more weakly developed than that of *D. latus*, which consists of well developed muscle-bundles.

The testes are very irregularly in the medullary field, either in a single row or in two rows, one of which is directly or obliquely ventral to the other.

The eggs are operculated, oval in shape, similar to but slightly smaller than those of *D. latus*, measuring on an average 58$\mu$ in length and 39$\mu$ in breadth; the lengths varying from 52·7$\mu$ to 67·3$\mu$, and the breadths from 36·5$\mu$ to 41$\mu$.*

From the above description, it is clear that our specimen belongs to the species *D. parvus* Stephens. Stephens gave five special characters as distinctive of his species, and the writers incline to concur in his conclusions. His main distinction is, however, based upon the external characters, especially the size of the worm and of the eggs. When identifying parasites, the size of the worm and of the eggs alone is not always sufficient to distinguish the species. In the course of his work on *D. mansoni*, Yoshida found that the adult form of this species varies greatly in size according to the host or other conditions of parasitism. The adult worms obtained from dogs fed with the liguloid larvae from the human host and from snakes measure 2 to 3 metres in length, while the worms from young cats fed with similar liguloid larvae from snakes and frogs are very small, measuring only 30 to 40 cm. in length. During recent years, Japanese investigators have shown experimentally that the tape-

* The writers propose in another paper to compare the internal anatomy of the closely related species, *D. latus*, *D. decipiens* and *D. mansoni*, in regard to which some confusion exists.

worms developed from the liguloid larvae of human beings, frogs and snakes are all the same species, namely, *D. mansoni*. This identification, however, is based chiefly upon the developmental study and external morphology of the worm, and not upon the details of internal structure. Thus, much work remains to be done on the internal morphology of the parasites in order to distinguish *D. latus* from *D. parvus*, and to determine finally whether the liguloid larvae of man, frogs and snakes develop into one and the same species or not.

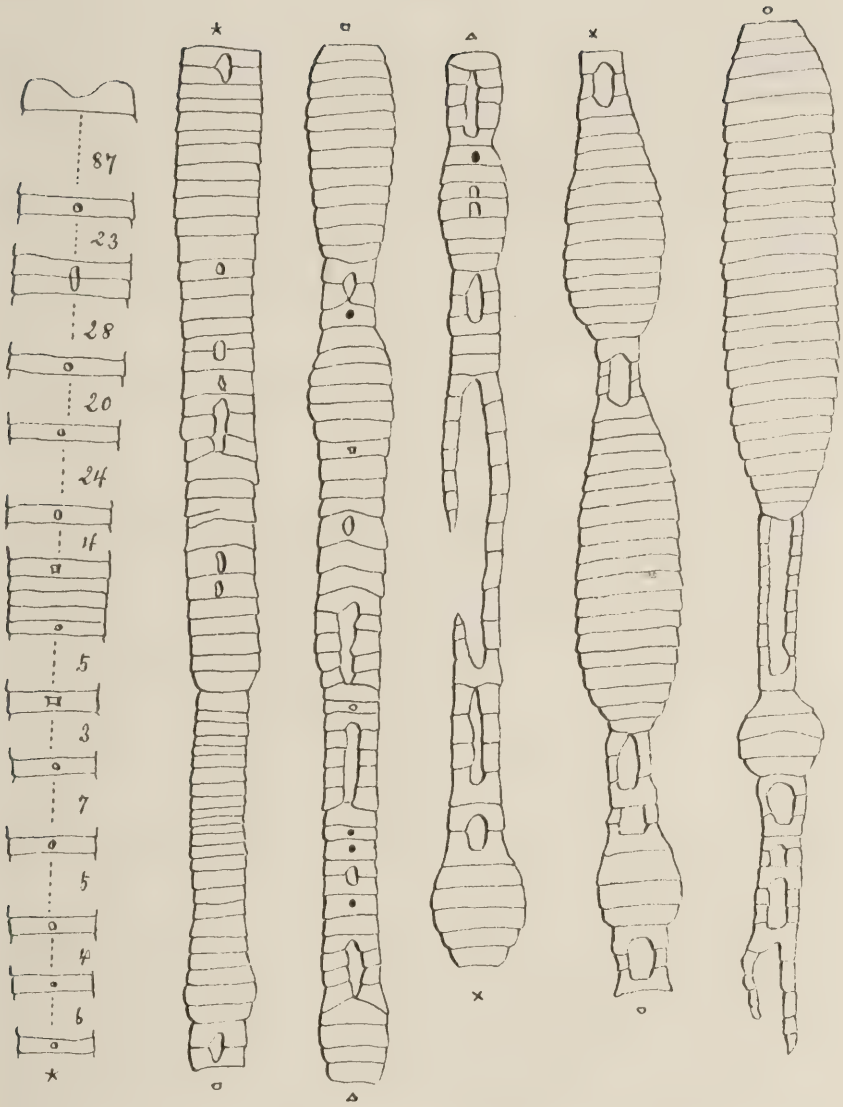
Some Japanese authors are of opinion, from a study of the liguloid larvae, that *D. mansoni* may be identical with *D. decipiens* of the cat. The two forms, however, have not yet been absolutely identified, as a precise description of *D. decipiens* is lacking, especially as regards the size and shape of the eggs. For the same reason, it is not possible to make a satisfactory comparison of *D. decipiens* and *D. parvus*.

## 2. MALFORMATION OF *DIBOTHRIOCEPHALUS LATUS*

This abnormal specimen was also presented by Mr. Nakasuga, who obtained it from a man aged about 50 years. It is a piece of strobila of 1·1 metres in length, proglottides being four hundred and eighty-eight in number, and the maximum breadth 13 mm. It presents remarkable abnormal segments, especially numerous in the posterior half of the specimen. The posterior end is bifurcated, each limb consisting of six segments. A few wedge-shaped segments are found in the anterior part of the strobila. The most pronounced abnormality is the fenestration which occurs in over forty segments. The largest one is 34 mm. long and stretches over ten segments, one side of it being discontinuous. The second largest fenestration is 22 mm. long covering eight segments, the third 9 mm. long over four segments; then come two fenestrations 8 mm. long over five segments, four 6 mm. long over four segments, five 5 to 5·5 mm. long over three segments, four 3 to 4 mm. long over two or three segments; the others are all rather smaller and are situated on a single segment or between two segments (fig. 1).

In some segments, the uterine loops become enormously enlarged to form a globular tubercle on one side of the worm, while the

Fig. 1.




Semidiagrammatic figure × 3/2. Figures between the segments on the left denote the number of segments without a perforation.

corresponding portion on the other side presents a depression of varying depth. In other segments, the depression becomes deeper and deeper and ultimately causes the fenestration, owing to the removal of the uterine mass. All fenestrations of the present specimen are invariably situated along the median line and represent the position of the uterine masses in the normal segments. In view of the foregoing facts, we consider that this fenestrated abnormality is probably caused by the disintegration of the uterine portion of the segment and the fusion of adjacent fenestrations with each other.

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## EXPLANATION OF PLATE I

Left. *Dibothriocephalus parvus*

Right. Malformation of *Dibothriocephalus latus*

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# A PIG NEMATODE, *GNATHOSTOMA HISPIDUM*, Fedchenko, AS A HUMAN PARASITE

BY

K. O. R. MORISHITA

*Department of Medical Zoology, The Kitasato Institute for Infectious Diseases, Tokyo, Japan*

(*Received for publication* 13 *January*, 1924)

Recently Dr. M. Kinoshita, of the Surgical Clinic of the Tokyo Imperial University, brought to me for identification a small piece of fresh human cutaneous tissue containing a parasite. Unfortunately, the nematode, which I was able to remove easily from the tissue, had been mutilated, apparently during the operation. There still remain, however, enough points of systematic importance to enable me to identify it with certainty as *Gnathostoma hispidum*. At least four cases of *Gnathostoma* in man have hitherto been reported, but they are all referred to *Gnathostoma spinigerum*, Owen. This paper presents, therefore, the first case of *Gnathostoma hispidum* in man.

*Gnathostoma hispidum* is a parasite generally found lodged in the stomach of wild and domesticated pigs, and has been found only once in the fat layer of a cow, in Berlin. In Japan, excluding Formosa, there have come to my knowledge only five cases of the occurrence of this species in the stomach of the domesticated pig, and this is the only species of *Gnathostoma* hitherto found in this

country. The new case reported here is of especial interest, not only because it concerns *Gnathostoma hispidum* as a human parasite, but also on account of its pathogenic properties.

## PATHOGENICITY

The patient is a male Japanese, 43 years old, who has lived long in Tokyo. On the evening of May 12th, 1923, he suddenly felt a slight pain on the left thenar eminence. He found a linear swelling of about 1 cm. there, and its length increased during the same and the following days to about 5 cm., with continued pain. On the third day the length of the swelling was about 8 cm. The patient then consulted Dr. Kinoshita, who recognised typical clinical features of the creeping disease, *Dermatitis linearis migrans*, in the affected part, and at the end of the progressive linear swelling, a small black object through the skin. On the same day Dr. Kinoshita excised the affected region to the length of about 2 cm., including the black object, and quickly brought it to me. This black object proved itself to be the intestine of the parasite, containing blood probably derived from the host. After the operation the symptoms disappeared. The worm was, therefore, the cause of the creeping disease.

## DESCRIPTION OF THE WORM

The worm is a young female. The head and anterior body wall are lacking. The posterior part of the body is cylindrical, tapering very gradually posteriorly to the abruptly rounded end. From the anterior end of the oesophagus to the posterior end of the body is about 5 cm., and the broadest part of the portions of the body which remain measures 0·51 mm. There are many fine transverse striations and small annulations on the cuticle, at intervals of 22$\mu$ to 33$\mu$. Small spines cover the whole body. The rows of the spines do not always agree with the annulations of the cuticle, as in the anterior part the intervals between the spine rows are 7$\mu$ to 10$\mu$. The spines are simple and directed posteriorly. In the anterior region

they measure 6$\mu$ to 7$\mu$ long by 2·4$\mu$ to 3·6$\mu$ broad at the base and become smaller posteriorly, measuring in the hindmost part only 3·6$\mu$ long by 2·4$\mu$ broad at the base, although large ones may occur at times.

The rows of spines are generally close together anteriorly and more separated posteriorly, and the spines of the same row are correspondingly more separated posteriorly, until finally they no longer form rows but are scattered irregularly and appear as dots. Ventrally the spines extend to about 74$\mu$ from the posterior end, and dorsally more close to the latter. The lateral lines are apparent, and the somatic muscle cells are spindle- or tadpole-shaped.

The one cervical sac which has fortunately been left is cylindrical, measures 0·4 mm. long by 50$\mu$ broad in the distal region, and is slightly constricted at 0·125 mm. from the distal end. A narrow duct is seen running through the entire sac.

The oesophagus is thick and club-shaped, measuring about 1·2 mm. in length; its maximum breadth, which lies in the posterior region, is 0·027 mm. Its lumen is lined by a triradiate cuticular wall. The intestine is of about equal breadth throughout, and only narrows slightly in the most posterior part. Its breadth is 0·135 mm. at the commencement, 0·22 mm. at the broadest point lying before the middle, and 0·115 mm. in the posterior end. The wall of the intestine consists of pentagonal or hexagonal epithelial cells. The rectum is conical and about 0·15 mm. long. The anus lies at the distance of 0·08 mm. from the posterior end of the body. Owing to shrinkage, the tail is seen as a conical process. As to the genital organs I have nothing to note.

The foregoing is all that I have been able to ascertain in my specimen. Although it is incomplete, the presence of cervical sacs and the mode of arrangement of the body spines conclusively prove it to be *Gnathostoma hispidum*.

I have still two other cases to note, which also refer to *Gnathostoma* in man. Both are from China. With the four generally known ones we can, therefore, count seven indubitable cases of *Gnathostoma* in man, and all are restricted to Eastern Asia, *i.e.*, Siam, Malay States, China and Japan. The cases from China will be reported on in another place.

## CONCLUSIONS

1. A nematode newly recovered from man in Japan is *Gnathostoma hispidum*, Fedchenko.

2. This is the first recorded case in which this species occurs as a human parasite.

3. The worm caused a typical creeping disease.

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I am indebted to Professor S. Goto for his kind supervision, and to Dr. M. Kinoshita for kindly placing the material at my disposal; my sincerest thanks are due to both of these gentlemen.

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# *GASTRODISCUS HOMINIS*

BY
CLAYTON LANE

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Under the heading '*Gastrodiscoides hominis*,' Leiper (1923) wrote thus: 'The systematic position of this parasite, found first by Lewis and McConnell in 1876, has been the subject of consideration by several workers. Placed in the genus "Amphistoma" by the discoverers, Fischoeder, in 1902, on account of the flattened ventral aspect, transferred it to the genus *Gastrodiscus*, and in 1913 Leiper made it the type species of a new genus *Gastrodiscoides*. The validity of the separation of the species from other forms, *G. aegyptiacus* and *G. secundus*, met with in the horse in Africa and India respectively, has been questioned by Lane (1922), but has been supported more recently by Khalil (1923).'

The characters of *G. hominis* selected by Leiper (1913) as justifying its generic separation were:—

(1) Large genital cone.
(2) Position of genital orifice.
(3) Smooth ventral disc.
(4) 'Testes tandem.'

The reported examinations of *G. hominis* are the following:— Lewis and McConnell (1876) dissected specimens obtained by autopsy from man; Stephens (1906) examined by section material collected by Ross in Assam; Leiper (1913) reported examination by section of material collected from man by Wise in British Guiana, and by Mackie in Assam; Lane made complete transverse and sagittal serial sections of worms from Lewis and McConnell's original material received through the courtesy of Sir Leonard Rogers, in effect type material, although no type specimen had been designated by Lewis and McConnell. With Low (1923), he then reported in condensed form the results of his reconstruction, having, as a preliminary, demonstrated one of the sections at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene

(Lane, 1922); Khalil (1923) described material and illustrated a squash preparation harboured by the Napu mouse deer ('*Tragulus napu*') from the Malay States, which died in the gardens of the Zoological Society of London; Maplestone (1923) re-examined Stephens's sections.

These descriptions have dealt with Leiper's four generic characters as follows:—

(1) Stephens makes no mention of a genital cone. In Leiper's specimens it was markedly present. In Lewis and McConnell's collection there was, as Lane's sections showed (Lane and Low, 1923), no cone but a depression surrounded by a tuberculated area encircling the opening of the genital atrium, a structural arrangement to which Lane (1923, p. 1693) gave the designation of genital pro-atrium. At this spot, as was indicated, the subcuticular sheet of muscle is incurved as a dome surmounting the genital pore; this on contraction and flattening would clearly evert the genital pro-atrium and produce a genital papilla. This dome of muscle, equally marked in longitudinal or transverse serial sections, Lane designated as a pseudo-sucker. He emphasised the fluke's affinities with *Watsonius*, already noted by Leiper ten years earlier, by placing both genera in a new tribe Gastrodiscidi, one of whose features was this same muscular dome, which Stiles and Goldberger (1910) had described for *W. watsoni* as 'a well-defined, curved (with convexity dorsal), muscular layer.'

Khalil distinguished a genital cone in all the fifty specimens he examined, and considers Leiper justified in taking a cone as one of his differentiating points for this genus. No amount of negative evidence, however, can invalidate a single positive observation; no matter how often a genital depression may be absent, its authenticated presence eliminates it inevitably as a valid generic character; and a genital depression is a marked feature of Lewis and McConnell's 'type' material.

Maplestone (1923) next gave two illustrations of Stephens's material. In one fluke there is a marked genital papilla, in the other there is none. Further, he illustrates *Cotylophoron cotylophoron*, *Brumptia gigas* and *Paramphistomum explanatum*, showing how, in each case, the genital opening may lie at the summit of a papilla or at the bottom of a depression, and writes of *G. hominis*,

'These two drawings, taken in conjunction with Leiper's figure, indicate that the presence or absence of a prominent genital papilla, or of a genital atrium, are purely matters of chance, and are of no more diagnostic value in this instance than in any other species of the group *Amphistomata*.'

The evidence, therefore, is that *G. hominis* may, in common with other Amphistomata, show either a genital papilla or a genital depression, and that it possesses certain anatomical muscular dispositions clearly capable of producing these alterations in the fluke's profile.

(2) Leiper does not elaborate this generic distinction by which *G. hominis* differs from other members of the genus *Gastrodiscus* beyond saying that it lies in 'the nearness of the genital pore to the edge of the ventral disc-like expansion.' It may be considered in greater detail. In *G. hominis* this pore lies about the middle of the anterior portion; in *G. aegyptiacus* about the anterior margin of the disc; in *G. secundus* well within the disc. *G. minor*, the other species of this genus, is practically a *nomen nudum;* its original designation (Leiper, 1913) never having been amplified; Maplestone treats it as a synonym of *G. aegyptiacus*, and it need not further enter into these considerations. But in *G. hominis* where does the disc begin? Stephens noted that neither anteriorly nor posteriorly did his specimens have a complete rim to the disc. Leiper characterised this statement as not accurate, since the rim 'always is sufficiently well marked to distinctly separate off the anterior conical portion of the worm from the disc.' Of the three specimens which Lane illustrates, the anterior rim of the disc is ventrally marked in one, indefinite in one, absent in one. Khalil found the distinction between disc and cone indefinite. But if the anterior margin of the rim be ventrally an inconstant structure, the line of junction of cone and disc may be roughly established by the lateral and dorsal aspects. Thus judged, Lane illustrates in Lewis and McConnell's material the anterior portion as occupying 3·5 out of 7 mm.; Khalil as 2 out of 7 mm. The anterior edge of the disc is then a point of varying position and particular indefiniteness in *G. hominis*, and one not affording a basis for generic distinctions. Moreover, if the position of the genital pore be considered relative to the viscera, if in accordance with modern usage the internal

anatomy be substituted as a systematic basis for the unsatisfactory one of external appearance, the pore in *G. aegyptiacus* and in *G. hominis* lies about opposite to the middle of the oesophagus, in *G. secundus* posterior to the oesophageal bifurcation. It would not be more arbitrary to suggest this as a generic character than the one based on the superficial shape, but Maplestone has shown that the position of the genital pore in front of or behind the gut fork is not even of specific value in this group.

(3) No one has examined the prominences found on the ventral surface of the disc of *G. aegyptiacus* so minutely as Looss. He described, in addition to the ordinary body musculature and beneath and parallel to the ventral surface of the disc, a network of very strong muscular fibres, the strands crossing at right angles and interlacing where they cross. He points out that general contraction of the fluke will force the parenchyma through the meshes so formed, and that local contraction of the fibres is to be looked upon as capable of producing a like effect. There exists, then, an anatomical disposition of muscle, and upon that disposition and upon muscular contraction does the presence of these eminences depend. They are prominent or replaced by depressions—with intermediate stages—according to the general and local state of muscular contraction.

It scarcely requires reiteration that neither genus nor species can rest on shape induced by muscular contraction.

(4) 'Testes tandem.' Stephens has shown that what Lewis and McConnell judged to be the ovary was really the posterior testis. The original observers accordingly found the testes tandem, as did Stephens, Leiper and Lane. In Khalil's material, surely preserved under ideal conditions, the illustration of a squash preparation, which he accepts in his description as generally applicable, shows them strictly diagonal. The first writers used dissections, the next three reconstructed serial sections, the last both squashed and sectioned flukes. Furthermore, in the illustrations of Lewis and McConnell, Stephens, and Khalil, both testes are in the disc; in those of Leiper the anterior testis lies at the junction of the two portions, but extends further into the cone than into the disc; in Lane's, the anterior testis is far in the cone if one judge by the dorsal margin; in the disc, if one judge by a possibly significant ventral depression, it being a matter of

arbitrary personal opinion as to whether it be judged to lie in cone or disc. Khalil considers 'that Leiper's definition of the genus ought to be modified as regards relative position of the testes.' The position, however, seems to be as follows:—The anterior testis may in undistorted specimens lie in the disc or more or less in the cone, the other testis lying axially behind it; in squash preparations the other testis may readily be made to take an oblique position. In other words, their relative position is alterable with methods of examination, and cannot be made a generic character.

It appears, therefore, that the genital papilla, the ventral prominences, the relation of the genital pore to the anterior edge of the disc, and the position of the testes are data insufficient to justify the creation of the genus *Gastrodiscoides*.

The work of Maplestone (1923) on this group, by the mass of observed fact on which it is based, stands apart from all that has gone before. Where others have proposed a new species or genus upon a single specimen, not infrequently immature, he has shown by the examination of hundreds that full gradations exist between different supposed species so that but one exists, and has made a very weighty addition to the existing protest against the use of the shape and size of muscular structures as of specific or generic value. He has shown, among other things, that the generic differences which induced the separation of *Watsonius* from *Pseudodiscus* are, on analysis, untenable, so that the valid name of man's parasite will become *Pseudodiscus watsoni*. But the considerations which he adduces, based on these hundreds of examinations of various species, cannot evidently be limited to the group concerned; they must have a general applicability for trematodes. For instance, the specific differences between *Euparyphium malayanum* and *Eu. suffrartyfex*, in so far as they refer to the shapes of the flukes and, it may be added, to the relative sizes of the testes, are untenable, and the validity of the latter species will depend upon whether the number of necklet spines and the presence of large corner spines prove in this genus to be true unvarying specific characters.

Similarly his considerations show that while Leiper was correct in refusing to accept the effects of muscular action as a valid basis for Stephens' *Paropisthorchis*, he was incorrect and inconsistent in accepting them for *Gastrodiscoides*. They justify the already

advocated suppression of *Watsonius macaci*, Kobayashi, 1920, and *Heterophyes nocens*, Onji and Nishio, 1915, as synonyms of *W. watsoni* and *H. heterophyes*, respectively (Lane and Low, 1923). They must equally be used to eliminate those cestode species, in so far as they are based on overlapping measurements, whose diagnosis is such that it may be a mere matter of personal taste as to which species shall have the credit of harbouring an individual specimen.

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# SOME MORPHOLOGICAL FEATURES OF *PLASMODIUM FALCIPARUM*

BY

J. W. W. STEPHENS

(*Received for publication 22 January*, 1924)

PLATES II, III

W.S. admitted to hospital 7.10.22 from West Africa.

A microscopical examination of stained films (Leishman or Giemsa) showed numerous *P. falciparum* parasites and a very scanty crescent infection.

The red cell infection rate was approximately 1 per cent., and the relative numbers of single and multiple infections were :— single, 69 per cent. ; multiple, 31 per cent.

The large majority of the parasites were uniform in size, of a diameter approximately equal to a third of that of the red cell. No pigment was detected in any of these ' rings.' It was frequently observed that part of the cytoplasm stained a deep bluish black, either in the form of an isolated spherical dot or dots, resembling somewhat pigment grains, or in a streak not uncommonly extending from the nucleus ; this shade of colour contrasting sharply with the lighter blue of the rest of the parasite. The form of the nucleus, while variable, was commonly that of a half hoop or bar.

A more prolonged examination of these films revealed many peculiar forms, some of which are here figured diagrammatically.

These forms were somewhat uncommon, but could always be found by systematic search through the films with a mechanical stage.

They can be arranged in three groups.

PLATE II—

(*A*). *Those in which there is a cytoplasmic connection between two adjacent parasites.*

Figs. 1-4. The appearance is that of a bridge or strand varying in width, in some cases clearly visible ; in others (not figured) the apparent strands were so thin that doubt arose as to their reality.

(*B*). *Those in which there is a nuclear connection between two adjacent parasites.*

Figs. 5 and 6. The parasites overlap, and accordingly, the appearance of a nuclear connection may be accidental.

Fig. 6. The symmetry of the two unusually elongated parasites is noteworthy.

Figs. 7-9. Fine threads pass from one nucleus to the other.

Fig. 8. Whereas in Fig. 7, two parasites were evidently present, in this case it was impossible to be certain whether one or two separate parasites existed.

Fig. 9. Has the appearance of a dividing parasite, the red cell being spindle-shaped. No other example like this was found.

Figs. 10-16. Thicker easily visible strands stretch between the nuclei.

Fig. 10. In this case again, apparently only one parasite body was present, and the nuclear symmetry is striking.

Fig. 12. A parasite beginning to divide or a parasite with a bilocular ' vacuole.'

Figs. 13, 14, 16. The parasite bodies are distinctly separated from one another in each case and apparently are in process of division.

Fig. 15. Traction may have had an influence in producing this extended form.

PLATE III—

(*C*). *Symmetrical Parasites.*

Figs. 1-4. Although speaking generally, one ' ring ' is like another, in these cases the symmetry and correspondence in morphological detail would appear to be more than an accidental occurrence.

(*D*). *Miscellaneous forms.* These include a number of forms differing considerably in appearance from those described in the standard text-books.

Fig. 5. Has two symmetrical nuclei, a rare feature in the parasites in these films.

Fig. 6. A very peculiar form. Two overlapping parasites, one having a curved crescent-like appearance.

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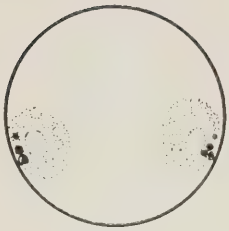

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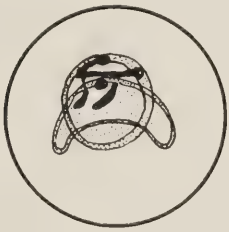

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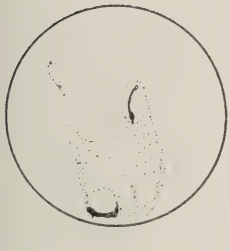

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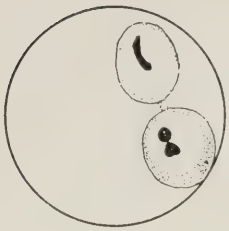

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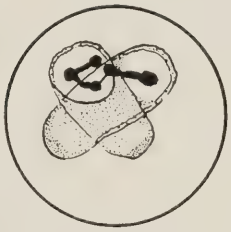

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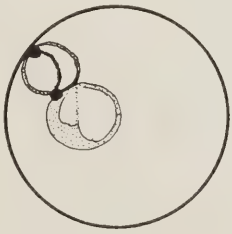

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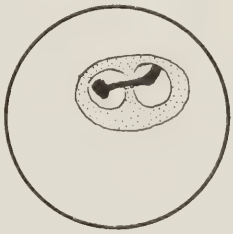

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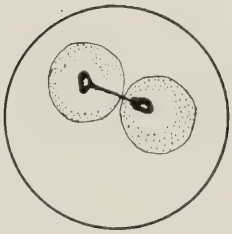

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Fig. 7. A large parasite form, staining intensely, with several nuclear masses, very unlike other ' ring ' forms.

Figs. 8-13. It is impossible to say in each case how many parasites are present.

Figs. 11 and 12. The appearances suggest plasmodial masses.

Fig. 13. The sharp-tailed ends are noteworthy.

Figs. 14-16. Extraordinarily irregular forms, in which it is impossible to distinguish how many parasites are present. Whether these forms should be called ' disintegrating ' or ' degenerating,' etc. seems questionable without further evidence.

# THE PEARL-INDUCING WORM IN THE CEYLON PEARL OYSTER

BY

T. SOUTHWELL

(*Received for publication* 26 *January*, 1924)

The Ceylon Pearl Fisheries are characterised by the fact that the harvest obtained from them is of a very irregular character. Occasionally, fisheries have been held every year for a number of years in succession, but the historical record of the pearl banks shows that numerous barren years, sometimes extending over long periods, have intervened.

As a pearl fishery yields considerable revenue to the Ceylon Government, the Colonial Office decided in 1900 that a thorough examination of the conditions existing on the Ceylon Pearl Banks should be made, the object being to ensure annual fisheries, if possible. On the recommendation of the Council of the Royal Society, Professor W. A. Herdman, F.R.S., accompanied by Mr. James Hornell as assistant, proceeded to Ceylon in December, 1901.

After an examination of the Pearl Banks, Professor Herdman returned in April, 1902, but Mr. Hornell remained in Ceylon to carry on the work.

In 1906, the Ceylon Company of Pearl Fishers leased the Pearl Fisheries from the Government, and the writer went out to Ceylon as Scientific Officer ; on the resignation of Mr. Hornell in 1907, he became Scientific Adviser and Inspector of Pearl Banks to the Company.

In 1908, Professor Herdman paid a short second visit to Ceylon, spending practically all his time on the Pearl Banks.

The Ceylon Company of Pearl Fishers ceased to exist in 1912, and the writer resigned his post in November, 1911.

The general work done by these officers and the results arrived at are not material to this paper, except in so far as they relate to pearl formation and the pearl-inducing worm.

Before proceeding to a discussion of this problem it is desirable to point out that pearls may be differentiated into three kinds, viz. :

(1) Excrescences or Blisters on the inside of the shell, caused by boring animals or other foreign bodies. This type of 'pearl' will not be considered further in this paper.

(2) Muscle-Pearls or Seed-Pearls. Small irregularly shaped pearls, usually occurring under the epidermis in 'the region where the muscle-attachment epithelium passes over into the ordinary shell-secreting epidermis of the mantle' (Jameson). These seed- or muscle-pearls were presumed by Herdman and Hornell to be formed round minute limy concretions which were called calcospherules. Seed-pearls are usually numerous, often in clusters, small, irregular in shape and situated in the vicinity of the muscle insertions.

(3) Cyst Pearls or Orient Pearls (the valuable pearls of commerce). Herdman and Hornell believed that in the majority of cases cestode larvae formed the nucleus of Orient pearls in the Ceylon Oyster, and that the adult form of this larva was *Tetrarhynchus unionifactor*, Shipley and Hornell, 1904, although Herdman later on stated that 'Cestodes, Trematodes and Nematodes are all concerned in pearl-formation.'

In order to understand the nature of the problem it is necessary to point out that the oyster shell is structurally composed of :—

(1) An outer layer called the Periostracum.

(2) A prismatic layer.

(3) The nacre, or mother-of-pearl, forming the bulk of the shell as well as the internal lining.

(4) The hypostracum, a substance secreted by a specialised epithelium and by which the muscles are attached to the shell.

(5) The hinge ligament.

The periostracum and the prismatic layers are secreted from the edge of the mantle, whilst the nacre, or pearly layer, originates from the whole of the outer surface of the mantle. The ligament is continuous with the periostracum.

The oyster is commonly infected with three principal larval parasites, viz.,

(1) A larval *Tetrarhynchus*, found in the wall of the gut (figs. 1 and 2).

FIG. 1. Older larval stage of *Tetrarhynchus* met with in the tissues of the pearl oyster's gut. After Shipley and Hornell. × about 12.

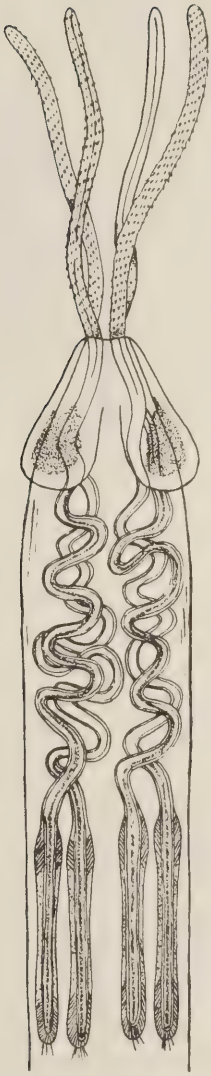

FIG. 2. Same, more highly magnified. After Shipley and Hornell. × about 50.

(2) A rather large (0·5 to 1·5 mm.) globular Cestode larva morphologically belonging to the genus *Tylocephalum*, found in the liver, etc. (fig. 3).

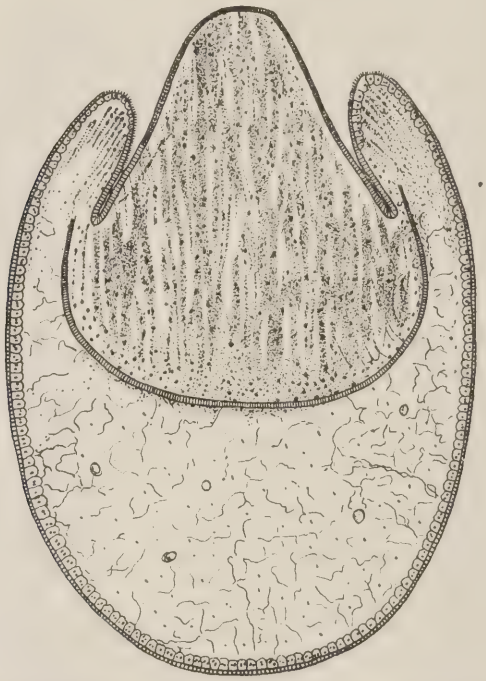

FIG. 3. Longitudinal section through the globular larva of *Tetrarhynchus unionifactor*, Shipley and Hornell, 1904. After Southwell. × 960.

(3) A smaller (about 0·1 mm. to 0·2 mm.) globular Cestode larva, morphologically belonging to the genus *Tylocephalum*, and also found in the liver, etc.

Herdman and Hornell believed that the *Tetrarhynchid* larva was simply a later stage of the larva in the globular cysts and that the adult worm occurred in different species of Elasmobranch fishes, which are known to feed on oysters. Herdman, however, pointed out that it was possible that the globular larvae (Nos. 2 and 3 above) might belong to the genus *Acrobothrium* ( = *Tylocephalum*). The adult *Tetrarhynchus unionifactor* has not, up to the present, been adequately described.

The distinction between these two types of larvae was clearly recognised by the present writer, who in 1910 wrote 'it would

certainly appear more probable as well as simpler for this larva (Nos. 2 and 3 above) to develop into a *Tylocephalum* (as is believed by Seurat) than into a *Tetrarhynchus*.'

The wide difference between the genera *Tylocephalum* and *Tetrarhynchus* will be evident from figs. 4 and 5.

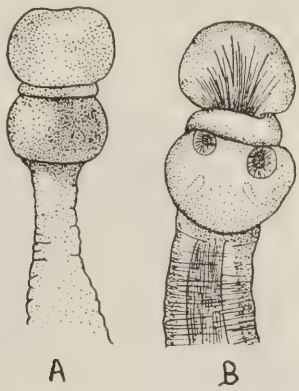


FIG. 4. *Tylocephalum pingui*, Linton, 1890. *A*—Head and neck of living specimen. × 18. *B*—Same when made transparent in clove oil. × 24. After Linton.

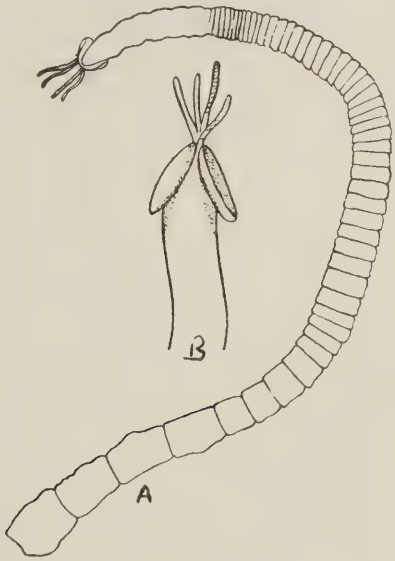


FIG. 5. *Tetrarhynchus unionifactor*. *A*—Entire worm. × 8. *B*—Drawn from life, showing fusion of the bothridia anteriorly, and the apical emergence of the proboscides. × about 25. After Shipley and Hornell.

A cyst pearl is almost invariably formed round a nucleus. This nucleus is believed to set up local irritation which results in a migration of epithelial cells normally concerned in secreting the nacre of the shell to the offending particle which it surrounds as a globular pearl-sac. The particle is thus coated with successive globular layers (fig. 6). It is believed that pearl formation only takes place round larvae which have died for reasons unknown, and which accordingly set up local irritation.

Fig. 6. Pearl in gill of *Mytilus edulis* showing disorganised nucleus and a distinct sac. × 300. After Herdman and Hornell.

The larval forms which occur in the wall of the gut of the oyster are apparently of the same species as the *Tetrarhynchid* parasites found by the writer in *Balistes spp.* and *Lethinus spp.*, etc. The latter species of fish, and possibly other species of Teleosts, are probably as the writer pointed out in 1910, co-lateral larval hosts.

The globular cystic larva found in the pearl oyster is normally enclosed in a fibrous cyst and, as Herdman showed, it is clear that in this condition it cannot become the nucleus of a pearl. Pearl formation must commence before the fibrous capsule develops, and

there is obviously no reason why this should not occur. Several pearls enclosed in typical pearl-sacs were on many occasions sectioned and found to contain the remains of the larva normally occurring in globular cysts. Jameson (1912) points out that in *Mytilus* the worm nuclei found in the centre of the mussel-pearls are quite large (0·5 mm.) and easily diagnosed. In the Ceylon pearl oyster the pearl nuclei would naturally be very small, if pearl formation took place round larvae at a stage so young that the fibrous capsule had not developed round the larvae. It should be remembered, however, that the essential condition for pearl formation is an epithelial sac, not a nucleus.

Various investigators (Willey, Hornell, Southwell) found that the globular plerocercoid larvae were capable of multiplying endogenously and in this way the infection of the pearl oyster is increased.

Although oysters are, as a rule, heavily infected with these globular larvae, cyst pearls are comparatively scarce. The explanation offered, and it seems a reasonable one, is that it is only the dead larvae which set up irritation. Jameson maintains that the irritation is not mechanical but toxic.

In 1910 the writer, with a view to deciding definitely by experiment what the adult forms of the larvae found in the pearl oyster were, arranged to feed large rays with oysters and observe the result. An area of sixty-four square yards in the open sea at a depth of two fathoms was isolated by a network of expanded metal having a four-inch mesh. A few days later 36,000 oysters were placed in the bottom of the enclosed area. A large ray (*Taeniura melanospilos*) measuring 7 feet 6 inches, and a 'shark' (*Ginglymostema concolor*) measuring 6 feet 6 inches, were captured alive, and, after being treated with male fern extract and castor oil (in order to clear the gut of any parasites they might then have) were placed in the enclosure and allowed to feed on the oysters for twenty-eight days. At the end of that time they were killed and the intestines were found to contain the following parasites :—

| | |
|---|---|
| *G. concolor* : | *Tet. unionifactor*, 51. |
| | *Tet. herdmani*, 48 (some in stomach). |
| *T. melanospilos* : | *Tet. unionifactor*, 150 (in stomach only). |

The presence of *Tet. unionifactor* in a shark called for comment, as sharks were not previously known to feed on oysters.

The following year the experiment was repeated. Twelve thousand oysters were placed in the enclosure along with the following fish, which were first treated with male fern extract :—

1 *Taeniura melanospilos*, 4 feet 6 inches.
3 *Rhynchobatis djeddensis*, 5 feet.
1 *Ginglymostoma concolor*, 8 feet 7 inches.
2 *Trygon walga*, 6 feet 6 inches and 3 feet 7 inches.

The specimen of *T. melanospilos* died from the shock of transport and at the end of the third day all three specimens of *R. djeddensis* died.

There thus remained one *G. concolor* and two *T. walga*. As a check experiment two other rays (*Trygon spp.*) were trawled at the same place and at the same time ; one was killed and examined immediately and found to contain numerous cestodes in its spiral valve. The other was treated with 30 minims of male fern extract and killed after three days. Only a very few cestodes were found, but the numerous reddish indentations in the spiral valve clearly indicated the positions of those cestodes which had been dislodged.

Of the fish in the enclosure, one was killed after twenty-four days, and it was found that the parasites had not developed. The rest were killed after having been in the enclosure for forty-seven days.

The following list shows the Cestodes found in their intestines :—

| | |
|---|---|
| *T. walga :* | Small cystic forms only (undetermined). |
| *G. concolor :* | *Tet. unionifactor*, 38. |
| | *Phyllobothroides hutsoni*, n.sp., 140. |
| | *Phyllobothroides kerkhami*, 9. |

The writer does not claim that the experiments were in any sense conclusive. Cestode cysts occur in a large number of marine forms (jelly-fish, various small Teleosts, etc.), and it is quite likely that such forms gained entrance to the enclosure through the mesh. Whether they were eaten or not is open to doubt. The outstanding fact was that although *Tet. unionifactor* had never before been found by the writer in any sharks or rays trawled on the Pearl Banks (and large numbers had been examined over a period of four years), *Tet. unionifactor* was found on both occasions, in the fishes which had been kept in the enclosure and fed on pearl oysters. Further, it was remarkable that no specimen of the genus *Tylocephalum* was

found. That the fish had fed on oysters was evidenced by the fact that the sand at the bottom of the enclosure showed numerous fragmented oyster shells.

Jameson (1912) reviewed the work which had been done on the pearl-inducing worm in the Ceylon Pearl Oyster, and came to the conclusion that the globular larval cestodes found in the pearl oyster belonged to the genus *Tylocephalum*, and not to the genus *Tetrarhynchus*. As the larval forms of *Tylocephalum* occurring in the oyster are of two sizes, he named the larger form *Tylo. ludificans* and he associated this larva with an adult worm found by Hornell in the intestine of *Aetobatis narinari*. As pointed out by Herdman in a note at the end of this paper, the correct name of this parasite is *Tylocephalum unionifactor* (Shipley and Hornell, 1904). It appears to the present writer that *Tylocephalum ludificans*, Jameson, 1912, is identical with *Tylocephalum dierama*, Shipley and Hornell, 1906.

About six species of the genus *Tylocephalum* are now known, and although there can be little doubt that the larva is referable to the genus *Tylocephalum* it is absolutely impossible to say to which species the larva belongs.

The smaller larva Jameson named *Tylo. minus ;* no reference was made to the adult of this form which may, or may not, be synonymous with *Tylocephalum unionifactor*.

Jameson further remarked :

> 'I think there is very good reason to believe that Southwell did, in his feeding experiments actually transmit *Tetrarhynchus unionifactor* from the oyster to the *Elasmobranch*, but it is difficult to escape the conclusion that the worms found in *Ginglymostoma* were derived from the *Tetrarhynchus* larva found in or around the alimentary canal of the oysters and not from the globular *Tylocephali* (*sic*) in the other tissues . . . .'

Although it is undoubted that the globular larvae in the pearl oyster are *Tylocephala* and also that various species of *Tylocephala* have been found in rays (*Trygon spp.*), the most interesting question is why representatives of the genus *Tylocephalum* were entirely absent in the fish fed experimentally. The conclusion one is tempted to draw is that the specimens of *Tetrarhynchus unionifactor* were not developed from the globular cysts, but from the larval *Tetrarhynchids* found in the oyster.

Herdman (Report, Pt. V, p. 21) gives a series of figures showing the

hypothetical way in which a larva apparently belonging to the genus *Tylocephalum* might be transformed into a *Tetrarhynchid*. It appears to the writer improbable that such a transition takes place.

The entire absence of any species of *Tylocephalum* is absolutely unexplained unless one assumes that the globular larvae do actually develop into *Tetrarhynchids*, and this seems improbable.

Jameson, after examining 356 pearls derived with few exceptions from *Margaritifera vulgaris* chiefly from Ceylon, summarised his conclusions as follows :—

'(1) The evidence that the globular Cestode larvae, which Professor Herdman regards as the cause of the formation of "fine pearls" in the Ceylon Pearl Oyster, are a young stage of the worm described by Shipley and Hornell as *Tetrarhynchus unionifactor* is quite inconclusive. I consider these worms to be more probably referable to the genus *Tylocephalum* (or an allied form), and have, provisionally, described them under the name of *Tylocephalum ludificans* and *T. minus*, spp. nn.

'(2) The theory that these Tapeworms are the cause or a cause of the formation of pearls in the Ceylon Pearl-Oyster . . . . is supported by quite insufficient evidence, and even their occasional occurrence in the nuclei of Ceylon pearls has yet to be demonstrated . . . .

'. . . . It is, of course, possible that in certain of the Ceylon banks, conditions may exist which cause *Tylocephalum ludificans* to depart from its normal habit, and acquire an ectodermal instead of a fibrous cyst ; or it might even be found that in certain banks *another* species of *Tylocephalum* (or other cestode) occurs which, like the Trematode in *Mytilus*, normally and habitually gives rise to a pearl-sac in the tissues, and which has been confused with *Tylocephalum ludificans* . . . .

'(3) . . . .

'(4) The "Calcospherules," which Herdman identifies as the nuclei of muscle-pearls, are not free concretions, but are minute pearls formed of the hypostracum or muscle-attachment substance. They are, therefore, not the *cause* of the nacreous muscle-pearls, but a phase parallel to them. There is some reason to believe that the origin of muscle-pearls is associated with pathological invaginations or immigrations of the epidermis at the points where the muscle-attachment epithelium passes over into the ordinary outer mantle-epithelium.

'(5) Parenchyma-pearls (which name I apply to Professor Herdman's cyst-pearls) may be formed around grains of sand or other foreign particles, organic granular matter of doubtful origin, or bodies composed of varieties of the shell-substance which arise when the normal rhythm of secretion is disturbed (repair-substance). A foreign nucleus is probably rather exceptional. The ultimate factors which give rise to the epidermal sacs in which they are formed have yet to be discovered. Many of them are probably of the same origin as muscle-pearls, except that they arise singly at points where a few muscle-fibres are inserted into the shell, instead of in clusters at the regular muscle-insertions. The dark pseudo-nuclei of these pearls, which may easily be mistaken for the remains of the parasites, are usually composed of the repair-substances.'

Seurat, working on the parasites of the black-lipped Pearl Oyster (*Margaritifera margaritifera* var. *cumingii*, Reeve) of the Gambier

Archipelago, came to the conclusion that pearl formation was due in that species of oyster to a parasite which Giard (1903) placed near the genus *Cyathophyllus*, Kessl (= *Acrobothrium*, Olsson = *Tylocephalum*, Linton).

Seurat, later (1906), correlated this larva with an adult worm found in the intestine of *Aetobatis narinari*, which he named *Tylocephalum narinari*. There is, however, nothing to show that the young form belongs to that particular adult. The point of interest in these observations is the fact that Seurat believed that the globular larva was connected with pearl formation in *M. margaritifera* var. *cumingii*, Reeve.

Hornell (1922) summarises his later opinions on the question as follows :—

'The origin of these pearls has been a battlefield of theory in the past ; the resultant confusion appears to me to be due in large part to the lack of recognition that there are these two main categories of pearls, differing in origin, and that in the case of cyst-pearls the causative body may, and usually does, differ with the locality and the species investigated. In the case of certain mussels (*Mytilus edulis*) the causative nucleus has been found in certain beds in France, to be a larval trematode worm (Jameson and Boutan), and in certain fresh-water mussels in one locality this is replaced by a little commensal mite (Küchenmeister). In the case of the Ceylon and Indian pearl oyster, Professor Herdman and the author found it in many cases to consist of the dead body of a larval Cestode. To this we gave the name *Tetrarhynchus unionifactor*, and we correlated it with an advanced larval Tetrarhynchid of typical form found, commonly, encysted in the walls of the oyster's intestine. At a later date we discovered that the adult of the latter worm is found in the sexually mature condition in the intestine of an oyster-eating ray, *Rhinoptera javanica*. At one time we intercalated an intermediate host, one of the file-fishes (*Balistidae*) but, eventually, the species found in the file-fishes was found to be of a distinct species, not parasitic in the larval condition in pearl oysters. I have, however, come now to the conclusion that the spherical cestode larva found in abundance in the tissues of the pearl oyster and frequently as a nucleus in cyst pearls from the same mollusc, is not a younger stage of the undoubted *Tetrarhynchid* larvae encysted in its intestine. Possibly it is the larva of some species of *Tylocephalum* or other closely related genus, but this is a subject for further investigations.

'Few pearl oysters are free from this parasite. Usually the gills contain hundreds, often very minute . . . . The digestive gland is another favourite location for these cysts, opalescent white spheres conspicuous in the dark green of the gland.'

Hornell showed

'Two nuclei which I obtained by decalcification of small orient pearls ; there can be no question as to their identity with the spherical larvae found alive in the tissues. Neither Professor Herdman nor I ever claimed that all cyst pearls have such nuclei ; we recognised that other foreign bodies, notably grains of sand, occasionally function as the intrusive irritating factor and become pearl nuclei. We have also even found a small nematode worm, coiled upon itself, forming the nucleus. So far we went sixteen years ago. Subsequent investigation

shows me that a further qualification is necessary whereby cyst pearls may be divided into two sections, the one comprising pearls induced by the irritation of foreign bodies and the other those with nuclei of periostracal-like substance derived from the oyster's own tissues. The former class comprises, according to my investigations, the majority of the larger cyst pearls, the latter of the smaller ones of this description, which, as I have indicated above, constitute by far the larger proportion of cyst pearls. This conclusion of our local researches disposes satisfactorily of certain objections levelled at the cestode theory, and places the latter in its proper perspective ; we see that cestode larvae, though less frequently the cause of pearl formation than was at first believed, are nevertheless the *most important factor in the production of the larger and finer of Orient pearls* and, therefore, of supreme importance from the economic and commercial view-point. Let us now see how pearl formation proceeds in cyst pearls formed around intrusive foreign bodies (*b*) . . . . (*c*) . . . .

'Some of my earliest experiments made in Galle in 1902, have direct and fundamental bearing on this problem. These were in respect of the power of the oyster to repair injuries to the shell. They resulted in demonstrating that epithelial cells are capable, at least over the nacre-secreting area, of an alteration in the character of their secretive power upon emergency. Thus I found that if a fragment of shell in the centre of the valve were removed, exposing the mantle which, previously, had been engaged in secreting nacre, the first repair substance formed was not nacre, but a yellow parchment-like material apparently identical with periostracum. Only after a stiff layer of this was formed, was there a resumption of nacre secretion. Now in all the pearls I have examined and, notably, in button pearls formed after the old Chinese method, and within recent years refined and extensively employed on a commercial scale by the Japanese, I have found that the nucleus, whether it be a cestode larva, a grain of sand, or a spherule of mother-of-pearl (as in the Japanese culture pearls), is not over-laid directly by a nacreous layer, but has interposed between its surface and the eventual layers of nacre, a distinct and well-marked deposit of stiff yellow membrane identical with repair periostracum, which, indeed, it is. It is evident that the intrusion of any body into the ectoderm must affect it in a similar manner to that caused by a direct injury, such as a fracture of the adjacent shell would do; hence the impulse of the cells around the intrusive body is to pour out the primary secretion employed to meet such an eventuality. The inmost layer of such a pearl is invariably of periostracum. Only after the effects of the shock have passed and normal conditions are restored, does the nacre secretion begin to be again deposited. What seems to me to be the explanation is that the membrane repair substance is really the conchyolin basis of nacre with the lime salts withheld. In other words, after a shock, the epithelial cells intermit the secretion of lime salts, but continue the secretion of conchyolin, thus giving a periostracal appearance to what would normally be a nacreous layer (conchyolin + carbonate of lime).

'Another deduction which I have made from the investigation, is that only dead or dying parasites excite irritation of the character necessary to induce pearl formation. A living parasite does not irritate the tissues in the same way ; indeed, it merely induces the formation of a tough connective tissue sheath or cyst enveloping it wherein it lies quiescent and harmless, giving no further irritation. But in the case of a parasitic larva that arrives in the epithelium in a dying condition, exhausted or perhaps smothered in the secreted fluid poured out by the epithelial cells, a different situation is found. Instead of being within a layer of connective tissue, it lies in a depression of the epithelial layer of cells and these act differently from connective tissue cells—with a correspondingly divergent result.'

In a private letter to the author, Hornell states that his

'Latest opinion is that the pearl larva which was first put down as a larva of a *Tetrarhynchid* Cestode and named in consequence *Tetrarhynchus unionifactor*, is not the larva of a *Tetrarhynchid* at all, but is the larva of a Cestode of some other genus—which is more likely to be *Tylocephalum* than any other. But I consider that the adult of this larva is not as yet identified.'

## SUMMARY

We may now summarise our present knowledge with reference to the so-called pearl-inducing worm in the Ceylon Pearl Oyster as follows :—

(1) Herdman and Hornell (1902 to 1906) found a number of globular cestode larvae in the tissues of the pearl oyster which they concluded were the principal causative agent in pearl formation. The larva was actually found to be the nuclei of several pearls examined by Herdman and Hornell. This larva (the adult form of which occurs in various Elasmobranch fishes) was named *Tetrarhynchus unionifactor* by Shipley and Hornell in 1904. Herdman gave hypothetical diagrams showing the manner in which he considered the globular larvae might become transformed into *Tetrarhynchids*.

(2) At least three different kinds of Cestode larvae inhabit the tissues of the oyster, viz. (i) a larval *Tetrarhynchid* in the intestines of the pearl oyster and (ii) two different sizes of globular larvae found in various parts of the tissues of the oyster, and belonging apparently to the genus *Tylocephalum*.

(3) Seurat (1906) concluded that the causative agent in pearl formation in the pearl oyster of the Gambier Archipelago was a Cestode larva belonging to the genus *Tylocephalum*.

(4) Southwell (1910 and 1911), as a result of feeding experiments, obtained *Tetrarhynchus unionifactor* (and other Cestodes) but no representative of the genus *Tylocephalum*, and concludes that the specimens of *Tet. unionifactor* were obtained from the larval form of *Tetrarhynchus* found in the oyster's intestine and not from the globular cysts. Why the adult of the larvae in the globular cysts was not obtained is not understood, and remains a matter of some significance.

(5) Jameson (1912) states that the globular larvae in the pearl oysters represent two different species of *Tylocephalum* and that the

theory that tapeworms are the cause or a cause of pearl formation in the Ceylon Pearl Oyster is supported by quite insufficient evidence ; he points out that the larval cestode always occurs in a fibrous sac, whereas an epidermal sac is necessary before a pearl can be formed and also that parasitic infection apparently bears little relationship to pearl formation.

It should here be noted, however, that pearl formation was only presumed to take place round larvae which for some reason or other had died very early on, and as a result set up local irritation.

Jameson sectioned a considerable number of pearls and was unable to find any trace of a Cestode parasite in the centre. He further concluded that the nucleus of Ceylon pearls consists of grains of sand or other foreign particles or organic matter of doubtful origin, or bodies composed of varieties of shell-substance which arise when, through any cause, secretion is disturbed.

(6) Hornell (1922) agrees that the globular cysts in the pearl oyster belong to the genus *Tylocephalum*, and states that *Tetrarhynchus unionifactor* is to be correlated with the advanced larval *Tetrarhynchid* commonly found encysted in the wall of the oyster's gut.

The difficulty of arriving at a definite conclusion in the matter will be evident from the foregoing, but the following points appear to be well-established :—

(1) That the globular larvae in the pearl oyster belong morphologically to the genus *Tylocephalum*, and probably *Tetrarhynchus unionifactor* is the adult of the *Tetrarhynchid* larva occuring in the walls of the gut of the oyster.

(2) The reason why no representative of the genus *Tylocephalum* occurred in the fishes which had been specially fed on oysters is unknown. Experimentally, the globular larvae appeared to develop into *Tetrarhynchids*. It is desirable that feeding experiments should be tried again, on a bigger scale, and for a greater length of time, in order to decide definitely whether the larva does belong to the genus *Tylocephalum* or whether, as Herdman suggested, the globular larvae actually develop into *Tetrarhynchids*.

(3) There is no doubt that the globular larvae do frequently occur as pearl nuclei and that the pearl formation round them only takes place when, for any reason, a young larva dies before a fibrous cyst is formed and sets up local irritation. The fact that a

pearl sac of epithelial origin occurs round such pearls is established beyond doubt.

(4) Whilst it appears to be true that these dead globular larvae are the primary cause of pearl formation it is probable that other bodies form the nuclei of pearls, such, for instance, as grains of sand, amorphous shell substance, dead organic particles, etc.

(5) It would appear that from the financial or commercial point of view the value of these fisheries depends not entirely on their regularity and magnitude, but also on the number of pearls contained in the oysters. It is not unreasonable to assume that the yield of pearls could be increased if numbers of the globular larvae in the oyster could be killed by artificial means, whilst the oyster was alive and young. After such treatment the oysters could be returned to a localised area in the sea and left to grow. The writer is well aware of the fact that operations of this kind would be difficult, but they are certainly not impossible.

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*NOTE added by Sir William Herdman, January*, 1924.

After consideration of the further investigations that have been carried out by Southwell, Hornell, Seurat, and others, during the last twenty years, I am now inclined to think that the globular cysts in the liver of the Ceylon Pearl Oysters which Hornell and I found in 1902 and regarded as larval stages of a *Tetrarhynchus* and which were formally described by Shipley and Hornell in 1904 under the name *Tetrarhynchus unionifactor*, are—as Mr. Southwell says in the present paper—more probably to be referred to the genus *Tylocephalum*. If that is so, by the rules of zoological nomenclature, the correct name of the parasite comes to be *Tylocephalum unionifactor* (Shipley and Hornell, 1904) in place of *Tylocephalum ludificans* suggested by Jameson in 1912.

Southwell's experiments at Ceylon in feeding fishes on pearl oysters are most important and should be repeated in order to test further the curious result that although the '*Tylocephalum*' cysts are most abundant in the oysters, the resulting parasites in the fish are chiefly *Tetrarhynchids*.

W. A. H.

Since the above paper was written, Professor Sir William Herdman has drawn the writer's attention to a paper in which Dollfus (1923) records the occurrence of Cestode larvae belonging to the genus *Tylocephalum* as nuclei of pearls in *Meleagrina occa*, Reeve, and *M. irradians*, Reeve. Dollfus states that the adult of the parasite is not known, but that the larva does not appear to differ from the globular larvae in the Ceylon Pearl oyster. These, undoubtedly, belong to the genus *Tylocephalum*. Dollfus considers that the *Tetrarhynchid* larva in the Ceylon Pearl oyster is quite different from the globular cyst which occurs in the same mollusc.

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# THE RELATIVE NUMBER OF MALE AND FEMALE CRESCENTS

BY

J. W. W. STEPHENS

AND

R. M. GORDON

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Most of the standard text-books contain little, if any, information on this subject, or at least what there is, is confined to some general statement unsupported by satisfactory data. A search through the literature, not an exhaustive one we admit, has furnished us only with two records.

Knowles (1919) gives the following table :—

TABLE I.

| Date | Total number of crescents counted | Macrogametocytes | Microgametocytes |
|---|---|---|---|
| 25.11.17 ... ... ... | 27 | 23 | 4 |
| 27.11.17 ... ... ... | 56 | 49 | 7 |
| 28.11.17 ... ... ... | 29 | 25 | 4 |
| 29.11.17 ... ... ... | 19 | 18 | 1 |
| 30.11.17 ... ... ... | 45 | 41 | 4 |
| 1.12.17 ... ... ... | 31 | 29 | 2 |
| 2.12.17 ... ... ... | 26 | 25 | 1 |
| 3.12.17 ... ... ... | 3 | 3 | 0 |
| 7.12.17 ... ... ... | 16 | 15 | 1 |
| 11.12.17 ... ... ... | 14 | 14 | 0 |
| 12.12.17 ... ... ... | 4 | 4 | 0 |
| 13.12.17 ... ... ... | 9 | 8 | 1 |

Although the number of crescents counted by Knowles is small, yet on all occasions he found the females in excess, and if we take the average of his figures we get the values, females 21, males 2, i.e., the females are ten times as numerous as the males.

We have compiled the following table from the data given by Abrami and Senevet (1920), but have omitted the figures they give showing the corrections for the statistical error, as the number of crescents counted was sufficient in most cases to exclude any large error due to this cause.

TABLE II.

Section I.

| Date. | Total number of crescents counted | Females % | Males % |
|---|---|---|---|
| 25.10.20 ... ... ... | 1500 | 52 | 44 |
| 26.10.20 ... ... ... | 1500 | 47 | 51 |
| 27.10.20 ... ... ... | 1500 | 41 | 53 |
| 28.10.20 ... ... ... | 1000 | 52 | 44 |
| 31.10.20 ... ... ... | 400 | 45 | 47 |
| 1.11.20 ... ... ... | 200 | 56 | 38* |
| 2.11.20 ... ... ... | 83 | 55 | 38 |
| 3.11.20 ... ... ... | 85 | 62 | 30 |

Section II.

| | | | |
|---|---|---|---|
| | 175 | 59 | 29 |
| | 1015 | 56 | 41 |
| | 400 | 71 | 23 |
| | 200 | 76 | 19 |

* The author's figure is 27·7, probably a misprint for 37·7.

The first section of the table refers to the same case counted on various days, and the second section to four separate cases. It will be noted that the percentages do not add up to exactly 100, as the authors found that in certain cases they were unable to decide whether a crescent was a male or a female.

If we now take the average of the figures in the first section we get the following values : Females 51, males 43 (1·2 to 1).

It is noteworthy that on three occasions the males were in excess.

In the second section of the table we find that the figures for the females and males vary considerably, the females, however, being always in excess. If we take the average, the figures are, females 65, males 28 (2·3 to 1).

The counts made by ourselves were on a series of thin blood films sent to one of us by Dr. E. A. C. Smith, Federated Malay States. It may be of interest to state that they were made from the blood of a case of general paralysis of the insane, inoculated with simple tertian malaria (*P. vivax*) on 11 July, 1923. Simple tertian parasites appeared in the blood on 25 July, 1923, and crescents on 4 August, 1923 (the source of the *P. falciparum* infection is obscure), and in nearly all the films were so abundant that 1,000 crescents could be easily counted. Our experience has been similar to that of Abrami and Senevet, that occasionally we were unable to decide whether a crescent was a male or a female. If the films were deeply stained there was a tendency to call females male, and in such films care was required in distinguishing them, but in less deeply stained films the distinction was well-marked.

The figure for these doubtful elements, which was certainly less than 5 per cent., we have omitted from our counts.

Five hundred crescents were counted in each film by each of us, the one not knowing previously the figure obtained by the other. The difference in individual counts ranged from 4 to 15 per cent. ; the average being about 10 per cent. This may be due to the fact that the proportion of females to males varied in different parts of the film, or may be due to a difference in interpretation.

The results obtained by us are given in Table III.

TABLE III.

| Date | Total number of crescents counted | Observer A. Percentage of | | Observer B. Percentage of | |
|---|---|---|---|---|---|
| | | Females | Males | Females | Males |
| 5.8.23 ... ... ... | 500 + 500 | 72 | 28 | 76 | 24 |
| 6.8.23* ... ... ... | 500 + 500 | 70 | 30 | 85 | 15 |
| 8 a.m. ... ... ... | | | | | |
| 6.8.23* ... ... ... | 500 + 500 | 73 | 27 | 88 | 12 |
| 3 p.m. ... ... ... | 500 | 75 | 25 | | |
| 7.8.23* ... ... ... | 500 + 500 | 72 | 28 | 76 | 24 |
| 3 p.m. ... ... ... | | | | | |
| 8.8.23* ... ... ... | 500 + 500 | 67 | 33 | 78 | 22 |
| 3 p.m. ... ... ... | | | | | |
| 9.8.23* ... ... ... | 500 + 500 | 66 | 34 | 76 | 24 |
| 3 p.m. ... ... ... | 500 | 68 | 32 | | |
| 10.8.23* ... ... ... | 500 + 500 | 68 | 32 | 78 | 22 |
| 3 p.m. ... ... ... | | | | | |
| 12.8.23* ... ... ... | 500 + 500 | 72 | 28 | 82 | 18 |
| 3 p.m. ... ... ... | | | | | |
| 14.8.23* ... ... ... | 500 + 500 | 66 | 34 | 74 | 26 |
| 8 a.m. ... ... ... | | | | | |
| Average ... ... | | 70 | 30 | 80 | 20 |

* Quinine hydrochloride grains 20.

From Table III we get the following average figures: for A, females 70, males 30 (2·3 to 1); for B, females 80, males 20 (4 to 1); and for A and B combined, females 75, males 25 (3 to 1).

Finally we summarize the observations in Table IV.

TABLE IV.

| Authority | Case | Total number of crescents counted | Average ratio Females / Males |
|---|---|---|---|
| Knowles (1919) ... ... | Blood films made from 1 case on 12 different days. | 279 | 10 / 1 |
| Abrami and Senevet (1920) | (1) Blood films made from 1 case on 8 different days. | 6268 | 1.2 / 1 |
| | (2) One blood film from each of 4 cases. | 1790 | 2·3 / 1 |
| Stephens and Gordon (1924) | Blood films from 1 case on 9 days | 10000 | 3 / 1 |

## CONCLUSION

In a case observed by us on nine separate days, female always outnumbered male crescents, the average ratio being three to one.

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## ADDENDUM

In a blood film made on 15.11.23 by Dr. Govadham, Central Provinces, India, and received by us on 31.3.24, 1,000 crescents were counted. The ratio of females to males was 750 to 250, i.e., 3 to 1.

# TROPICAL EAR

BY

The late J. M. FORSYTH, M.B., Ch.B. (Glasgow).

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PLATE IV

This condition is known in the Malay states as 'Singapore Ear,' but it is prevalent in all tropical or semi-tropical climates. In Calcutta it is called 'Calcutta Ear,' in Java 'Java Ear,' in Hongkong 'Hongkong Ear,' but the comprehensive term 'Tropical Ear' is, perhaps, more suitable.

So far as I can find out no definite attempt has been made to investigate the nature of this condition, notwithstanding the fact that it is very common among all classes, European as well as native.

During the past twelve months I have examined and made cultures from over a hundred cases.

'Tropical Ear' may be defined as :—A localised infection of the external auditory meatus which does not involve the middle ear, but sometimes, though rarely extends into it.

From the etiological findings it is possible to divide it into two main groups, viz. :—

I. Otomycotic.
II. Bacterial.

### Group I. OTOMYCOTIC

Isolated cases of fungus in the ear have been found by many investigators. Thus Castellani and Chalmers give the following :—

*Mucor pucillus*, Lindt, 1886 ; *Lichtheimia corymbifera*, Cohn, 1884 ; *Lichtheimea ramosa*, Lindt, 1886 ; *Saccharomyces ellipsoides*, Rhees, 1870 ; *Monilia rhoi*, Castellani, 1909 ; *Aspergillus flavus*, De Bary, 1840 ; *Aspergillus flavus*, De Bary, 1870 ; *Aspergillus malignus*, Lindt, 1889 ; *Aspergillus repens*, De Bary, 1870.

In the fifteen cases met with, fourteen were found to be due to an *Aspergillus*, and one was found to be a yeast. As the yeast infection differs so much from the others and is an isolated case,

it has been described under ' Accidental Infections ' in the bacterial group, though it belongs to the *Saccharomyces*.

Among the fourteen cases of Aspergillus Infection, four distinct colour varieties were found :

(1) Black. (5).
(2) Reddish brown. (1.)
(3) Mouse-coloured. (1.)
(4) Green in three shades ; Blue-green, Olive-green, Grass-green. (7.)

*Aspergillus niger* is larger than the other species, is more virulent, and grows more quickly.

*Green aspergillus* differs from *A. niger* in the following points :

Colour : (*a*) Blue-green ; (*b*) Olive-green ; (*c*) Grass-green. It has a more delicate structure. It has finer sterigmata. It is smaller. The diameter of the spores is from 3-4 microns. The growth soon withers and the colour fades, though the spores remain alive for long periods.

The different varieties, Blue-, Olive-, and Grass-green breed true.

*Brown aspergillus* culture has a reddish brown colour and spores 3 microns in diameter. Only one case seen. Breeds true on sub-culture. Otherwise has the characters of an *Aspergillus*.

*Mouse-coloured aspergillus*, except for the colour, does not differ from the brown variety. Breeds true on sub-culture.

Clinical Manifestations. The symptoms were the same in all fourteen cases, though the duration and severity varied slightly.

In the early stage itching and irritation of the meatus are the only signs ; later, deafness and a feeling of ' fulness ' in the ear become more and more marked ; later still the irritation increases to actual pain. On examination of the meatus the picture is fairly characteristic. If wax is present, the only indication is the presence of white caseous looking material, mixed with the normal brown wax. If wax is absent, the white furry growth can be clearly seen on the walls of the meatus, and sometimes on the drum itself. Later the canal becomes completely choked with this white caseous material and the brown wax disappears, showing that the fungus grows on the wax and absorbs it. If left undisturbed, this white caseous material becomes crinkled and crenated and looks very

like a mass of sodden desquamated epithelium, for which it is often mistaken.

Clinically, except for slight redness of the walls of the meatus and occasionally slight opacity of the drum, no evidence of disease can be detected, once the meatus has been thoroughly syringed and swabbed out. There is no tenderness of the auricle or auricular glands ; no fever or malaise ; and the slight amount of deafness is due to the mechanical blocking of the ear.

Treatment. As many cases do not come for treatment until the condition is far advanced, some difficulty may be met in clearing the meatus, which is choked with a thick growth of fungus. It may be necessary to pick it out piece-meal with forceps. But in the majority of cases, softening with pure hydrogen peroxide and syringing with hot water and peroxide will dislodge the main bulk of the growth. The meatus and drum can then be swabbed clean of the remaining portions, which sometimes cling tenaciously, especially in the anterior recess between the drum and meatal wall ; a strong antiseptic should be used in doing this, such as S.V.R. (rectified spirit of wine).

To all appearances the meatus is free from growth and the patient cured ; but a recurrence is likely unless a more systematic treatment is adopted.

The following routine is suggested :—

First day—

(1) Instil pure peroxide into meatus and allow to act for two minutes.

(2) Syringe with hot water and peroxide until bulk of growth is dislodged.

(3) Swab out with dry wool, paying particular attention to the anterior recess and the surface of the drum.

(4) Swab out with hydrarg. perchlor. 1 gr. to the oz. of S.V.R.

(5) Pack meatus with wool soaked in a solution of perchloride in spirit $\frac{1}{4}$ gr. to the oz.

Second and Third days.

Repeat above.

Fourth day.

Clean as before, instil drops of perchloride in spirit (1 gr. to the oz.) and pack with dry wool.

The patient should carry on the daily treatment himself for one week as follows :—

(1) Instil pure peroxide and allow to act for two minutes.

(2) Dry thoroughly and instil two drops of perchloride in spirit ($\frac{1}{2}$ gr. to the oz.).

(3) Pack with dry wool.

*Weekly Inspection* should be made for a month and treatment repeated as on the first day. As well as this, the patient should on two days in each week, instil drops of carbolic in glycerine (1 in 5). In persistent cases it is advisable to vary the antiseptic and the following have been found effective :-

(1) Proflavine in saline 1-1000, or 1-500. The ear can be packed with wool soaked in this. The only objection to its use is that it gives rise to great itching, for some days after.

(2) *Linimentum belladonnae* and *Linimentum opii* equal parts. This can be painted over the walls of the meatus ; or a pledget of wool soaked in it can be packed into the ear. The burning pain to which it gives rise, passes away in about half-an-hour.

(3) Zinc sulphate gr. 10, cocaine hyd. gr. 2, boric acid gr. 10, S.V.R. $\frac{1}{2}$ oz., aqua ad. 1 oz. This can be used as ear drops twice daily, three days in each week for a month.

*Prophylactic Treatment.*

(1) Keep the ears as dry as possible.

(2) Cleanse the ear daily with 3 per cent. mercurial soap.

(3) Pack the ears when bathing with wool and sterile vaseline.

The following illustrative cases are of interest :—

Case 1. A. J. 1st September, 1920. Complains of fulness and discomfort in the ear. Meatus completely blocked with thick white plug. Removed with forceps and syringing, when large perforation is seen in anterior sup. quadrant.

21st September. Meatus almost choked again. Black mould growing on walls, flowers and stalks clearly visible. Perforation unchanged. Complains of discomfort but no pain.

25th September. Mould still present. Flowers clearly seen. No symptoms.
29th September. Mould still present.
30th September. No mould visible. Perforation unchanged.
1st October. No mould present.
3rd October. Mould again visible.
5th October. No mould. Perforation smaller.
7th October. No mould. No symptoms. Perforation smaller but permanent.

The *treatment* adopted was that given above.

Case 2. A. H. 13th October, 1920. Both ears blocked with white macerated material. Complains of great discomfort and deafness.

25th October. Ears clear.

28th October. Fungus again present. No symptoms.

22nd November. No symptoms, but small amount of white fur in depths of meatus from which *Aspergillus niger* grew.

7th March, 1921. Small amount of fungus in both ears. Complained of slight irritation for two days previously.

The above case was one of a traveller who could only come for a day or two for treatment, with long intervals in between.

The *treatment* was on the lines already indicated.

Case 3. E. S. M. 18th April, 1921. Deafness and great pain in ear of one day's duration; history of discomfort and increasing deafness for past ten days. Meatus completely blocked with white caseous material. This was so tough that it had to be picked out with forceps before the meatus could be syringed out. Portions of desquamated epithelium came with it, leaving a raw red surface on the walls and an acutely inflamed drum. A minute perforation of the drum present. (Culture made).

Carbolic and glycerine (1-5) drops packed in, as inflammation too severe to apply spirit.

19th April. Meatus clear. Drum greatly inflamed, with white patch of fungus on it surrounding the perforation which is distinctly larger. (Culture shows *A. niger*).

White patch on drum carefully removed and whole ear swabbed out with perchloride in spirit. The perforation healed rapidly.

## Group II. BACTERIAL

From the etiological findings it is possible to divide this group into three sub-divisions :—

(1) Simple.
(2) Accidental.
(3) True Tropical Ear.

### SIMPLE INFECTIONS

Symptoms.

Irritation in the ear, feeling of fulness, slight deafness. As a rule the meatus is blocked with wax; or there may be a slight mucopurulent discharge, together with a sodden appearance of the walls of the meatus.

Etiology.

The culture media show the presence of a mixed infection, comprising *Staphylococcus albus*, and *S. aureus*, *Micrococcus catarrhalis*, large diplococci, *Bacillus coli*, occasionally a few streptococci. In one case *S. aureus* in pure culture was found.

TREATMENT.

This condition readily clears up under treatment. Peroxide drops, followed by syringing with warm antiseptic lotion, drying thoroughly, and finally drops of perchloride in spirit, the ear being packed with dry wool; one thorough cleaning as above is often sufficient. The patient should, nevertheless, use glycerine and carbolic drops, for three days.

## ACCIDENTAL INFECTIONS

In this group are included those rare infections that could not be grouped elsewhere. The following case was due to a *Saccharomyces* infection.

CASE 1. B. 26th October, 1920. Complaining of pain and discharge from the ear and slight deafness. Meatal walls covered with this mucopurulent material. Meatus inflamed; small yellow vesicles seen on walls and drum. Drum very much inflamed with a yellow patchy appearance. No bulging of drum and no appearance of middle ear infection. No history of sore throat. (Culture made).

27th October. Great pain during the night, which passed away gradually, meatus full of glairy muco-pus, pale yellow in colour. Large perforation of drum in anterior quadrant.

30th October. Much cleaner. Drum less inflamed but still shows the peculiar yellow parchy appearance. Perforation slightly smaller. Culture shows a pure growth of a yeast.

1st November. No pus; greatly improved. Yellow patches on drum still present.

3rd November. Improved. Perforation unchanged.

18th November. No pus or inflammation. Ear dry. Perforation much larger, has extended on each side into the yellow patches.

2nd December. Perforation smaller.

18th March. Perforation much smaller, but apparently permanent.

*Treatment.* The double liniment was used daily for the first week. After that, perchloride in spirit.

Case of mixed *B. diphtheriae* and *B. coli* infection.

CASE 2. E. 18th July, 1921. Complains of pain in the right ear and slight deafness. Meatus full of yellow pus slightly glairy in consistence, walls slightly inflamed, drum inflamed but intact, no evidence of middle ear infection. Anterior recess filled with fungus-like material.

27th July. Discharge copious; thin, glairy, pale yellow in colour. Meatus painful and swollen. The symptoms had been quiescent and only reappeared the previous day. (Culture made).

29th July. Discharge copious of a thin, glairy, colourless appearance. Pain gone, swelling less. (Culture shows presence of *B. coli* and a *Diphtheroid bacillus*). (Sub-culture made).

30th July. Discharge very copious. Pain gone; swelling less. (Sub-culture shows *B. diphtheriae*).

(Second sub-culture made). The second sub-culture showed pure growth of *Diphtheria bacillus.*

31st July. Copious, translucent, glairy fluid. Slight swelling but no pain.

1st August. Unchanged.

2nd August. Complained of feeling drowsy the previous day. Discharge less, swelling of walls of meatus almost gone. White membrane seen on drum and adjacent wall, filling anterior recess. (Previously obscured by swelling of walls). Membrane very adherent.

3rd August. Discharge less. Some of membrane removed by careful swabbing, but too broken up for examination. Improvement continued and on 6th August no sign of inflammation was left except a white mark on drum.

28th August. Recurrence of all symptoms which cleared up after a week's treatment, and did not again return.

*Treatment.* First day. Cleaned and packed with carbolised glycerine. Drops of glycerine and carbolic given for daily use. When the Diphtheria Bacillus was found, the ear was packed daily with perchloride in spirit (1 gr. to the oz.) and 4,000 units of antitoxin were given subcutaneously into the abdominal wall. This treatment made no impression on the quantity of the discharge, but the nature of the discharge changed slightly; the yellow tinge disappeared. The walls of the meatus and the drum were then painted with Proflavine 1 in 100 saline. This also had no effect.

1st August. Pure peroxide two minutes, syringing with warm water and peroxide; drying thoroughly; painting the whole interior with antitoxin and packing ear with wool soaked in antitoxin. Improvement was noticed at once. This was repeated for six days, by which time all signs of infection had completely disappeared. The patient was told to continue with the glycerine and carbolic drops for a day or two. Three weeks later the whole condition reappeared, but completely cleared up after a further course of treatment with antitoxin.

This was an interesting case as it showed a double infection with *B. coli* and *B. diphtheriae.* The *B. coli* disappeared under ordinary antiseptic treatment, while strong antiseptics such as perchloride in spirit (1 gr. to the oz.), pure peroxide, and proflavine 1 in 100, had no effect on the diphtheria bacillus. Antitoxin acted at once and with great effect when applied locally. As no perforation was present the infection did not come from the throat or nasopharynx; and repeated swabs from both these localities showed the absence of *B. diphtheriae,* proving that the infection was a primary infection of the external auditory meatus.

### TRUE TROPICAL EAR

The third type of bacterial infection is distinguished, firstly, by a constant group of clinical symptoms and, secondly, by its etiology.

Symptoms. Onset always acute. Malaise marked, fever between

99°-100°. Pain acute; unilateral, though other ear may become infected later.

The pain is centred in the external auditory meatus, but diffuses widely over the same side of the head and may give rise to a severe, throbbing headache. May be agonising in character, excluding all possibility of sleep. Together with the throbbing ache, sharp shooting pains are an alarming feature. Moving the jaw greatly aggravates the pain.

*Tenderness* a very marked feature, and usually involves the whole of the pinna, the anterior and the inferior auricular glands and sometimes the superior cervical glands. It is centred in the meatus where even the slightest touch is unbearable. No tenderness over mastoid (provided the pinna is not touched). The anterior and inferior auricular glands, and sometimes cervical glands are also involved. Pinna swollen. Walls of external auditory meatus swollen throughout its entire length, and as a rule the canal is obliterated by apposition of the walls. The walls are so swollen that it is often impossible to insert even a fine probe between them. But as acute pain is practically the first symptom, the patient usually comes for treatment before the swelling has obliterated the canal. No swelling over mastoid region.

*Presence of small vesicles.* Greenish-yellow in appearance on the walls of the meatus, which are thin walled and filled with yellow pus with a faint green tinge in it. When the vesicle is removed, a raw red surface is left. In the later stages it is possible to trace a minute sinus running down from each vesicle towards the cartilage of the pinna, from which pus can be seen exuding. The swelling is at first local, but it rapidly gives rise to intense inflammation, which causes swelling of the whole extent of the canal.

*The Drum* remains unaffected except for slight inflammation. In no case has perforation resulted.

*Course.* The condition runs the following course:—Infection of ear; formation of vesicles on walls of meatus; pain and tenderness and swelling commence. Infection quickly spreads into deeper zones; inflammation of the cartilage, which gives rise to intense pain; the swelling of walls of canal, which becomes acutely tender; tenderness of pinna and swelling of glands follow. The next stage is the formation of a deep-seated abscess, which does not 'come

to a head ' and burst, but burrows out a minute channel, which discharges the pus in minute quantities at irregular intervals, always leaving infection behind. Each time pus is evacuated the symptoms moderate and sometimes disappear altogether ; but in a few days, often at the end of a week even, they reappear and the whole condition flares up again. A chronic abscess and the sinus is formed eventually and this may go on to necrosis of the cartilage lining the meatus or forming the pinna, with sometimes sloughing of considerable areas of tissue.

The condition may continue for several months. This is due to the fact that the infection remains deep seated and even spreads along the deeper planes before coming to the surface ; when it does come to the surface it often reinfects another spot on the meatal wall. At no time, throughout the course of the disease, does pus appear in large quantities, as in middle ear infection.

It soon became evident that it was only when *B. pyocyaneus* was present, that symptoms of an intense character followed. And later it was found that in the majority of cases, the cultures showed almost pure growths of *B. pyocyaneus*, and in many cases, no other organisms were found even after prolonged search. The mixed infections usually included mild non-virulent organisms, such as *Micrococcus catarrhalis*, *Bacillus coli*, or large gram-positive diplococci. It was rare to find staphylococci or streptococci, thus differentiating the condition from middle ear infections and furuncle.

In the mixed infections, *B. pyocyaneus* outlived the other organisms, which were soon killed off by the antiseptic treatment. In the chronic cases where the pus came from a deep seated abscess, *Bacillus pyocyaneus* grew on media in almost pure culture. As the meatus is open to the outside air, it is not possible to make a culture which is uncontaminated, and, therefore, the results are all the more striking. In the hundred odd cases referred to, however mild the symptoms were, cultures were made. In many of these the medium remained sterile and no growth resulted, in the majority only slight growth of mild organisms appeared, showing that normally the walls of the meatus are more or less sterile. When, therefore, we find a virulent organism present, and associated with it, a constant set of symptoms not present in other infections, when we again and that in a large proportion of these cases, the infection with

*B. pyocyaneus* is a pure one, we must come to the conclusion that the germ is the cause of the disease; in other words, that *Bacillus pyocyaneus* is the cause of 'Tropical Ear.'

TREATMENT

*Mild Cases* include those which come early for treatment, some of which may in spite of treatment pass on into the severe stage.

If the meatus is choked with wax, it is necessary to syringe with warm water and peroxide; but unless it is absolutely necessary to clear the ear, do not syringe. This is an important point, because any moisture in the ear tends to make the walls of the meatus sodden, and a nidus is formed of softened dead epithelium, in which the germs propagate and through which they rapidly penetrate. After the ear has been cleansed and dried thoroughly, examine the walls of the meatus for vesicles; any found should be removed with forceps, and the pus absorbed on dry wool. Then instil a few drops of pure peroxide and allow to act for a few minutes, dry again carefully, and then swab the whole canal with perchloride in spirit. Finally, pack the ear with wool soaked in proflavine in saline 1-1000. A smart saline purge should be given and powders containing aspirin gr. 4, phenacetin gr. 4, caffeine citras gr. 2, taken thrice daily.

The best way to reduce the swelling and pain is to apply a pad of hot antiphlogistine, about half-an-inch thick, all round the ear and over the pinna, and keep it on all night.

Repeat the above treatment on the second day, but pack the ear with perchloride in spirit. If vesicles are present on the third day, swab out the whole canal with *Linimentum belladonnae* and *Linimentum opii*, equal parts. In ordinary mild cases the above treatment will clear up the condition, provided the patient uses peroxide drops followed by perchloride in spirit daily for another week, when the meatus should again be inspected and thoroughly cleansed, and then packed with wool coated with a mixture of *Ung. hydrarg. ammoniatum* and *Ung. acidi borici* in equal parts. If the condition does not clear up in a week, or recurs, it passes into the severe and then into the chronic stage.

*Severe Cases* always imply the formation of deep-seated abscesses. If the infection can be checked at once, as above, abscesses will not form, but once the germ penetrates into the deeper layers the above treatment will not avail.

If the canal is not completely obliterated by swelling, pack the whole canal with wool soaked in equal parts of *Ung. belladonnae* and *Ung. opii.* This is painful at the time, but the pain passes away in about half-an-hour. Apply a pad of very hot antiphlogistine about half-an-inch thick all over the ear and down into the entrance of the meatus. This should be renewed at the end of six hours, and in the interval heat should be kept applied by means of a hot-water bottle on the pillow. Give a calomel purge. Do not syringe the ear as the point of the syringe in the meatus causes great pain and the syringing serves no useful purpose, the infection being deep down in the tissues. Do not use peroxide for the same reason, and also because some of it will get caught behind the swelling, and with the formation of gas, will give rise to pressure and pain. The object of the treatment is to draw the infection to the surface where it can be dealt with, for it shows no tendency to come there itself. The whole difficulty in the treatment is to get at the infection and thoroughly eradicate it, the tendency for it to lie dormant for long periods (7 days in one case) being an added obstacle.

Operative Interference is found not only to be useless but dangerous as well, for the following reasons :—

(1) It is difficult to locate the position of the abscess in the deeper tissues, because the swelling involves the whole wall of the canal. When the abscess discharges pus it does so through a winding narrow sinus.

(2) If the knife does not strike the abscess, it opens up fresh tissues to infection.

Repeat the treatment given above on the second day. After the second day, the *pure carbolic treatment* should be employed at once, or if the meatus is completely obliterated on the first day, start with this method right away.

*Pure Carbolic Treatment.* A fine probe covered at the end with a very thin layer of cotton wool is dipped into pure carbolic acid and is used to touch the sides of all vesicles after they have been removed and the pus dried off. If a sinus is found, the exit should be cauterised and the probe passed down it as far as possible without causing bleeding. If, again, no vesicle can be located and at the same time the swelling of the walls of the meatus indicate the presence of a deep-seated abscess, the pure carbolic should be painted over

the most prominent part of the swelling on each wall. Care should be taken to avoid letting the acid overrun its allotted boundaries; this might produce severe burning or might injure the drum. But apart from this, large areas of meatal wall can be safely painted without fear of permanent damage. As much of the ear as possible should be dried carefully, for if the ear is moist the acid will diffuse over the ear and form a strong solution, which will injure the drum. After drying thoroughly, clean part with S.V.R. and allow it to evaporate. Then apply the pure carbolic with the tip of the probe moistened only, and paint the part two or three times till the surface turns white. Then pack the ear with dry wool. This is repeated the second day. On the third day it will be seen that the acid has killed the superficial epithelium, which can be readily removed, and the carbolic can then be applied to the exposed surface.

Gentle pressure should be applied to the swelling, but if pus does not readily appear, the pressure should not be continued, as it will force the pus along the lower planes and thus infect new tissue. If pus comes to the surface, it should be carefully wiped off, and care taken that it does not touch other parts of the meatal wall. Under this treatment the symptoms will rapidly disappear. The swelling goes down quickly, allowing of free access to the deeper portion of the meatus, which should be carefully cleaned each time; the pus will either come to the surface and discharge, or it will be absorbed. After the four applications, it is not as a rule necessary to continue with the carbolic. Treatment should, nevertheless, be continued for another week. The meatus should be cleansed with perchloride and spirit and the ear then packed with wool coated with *Ung. hydrarg. ammon.* and *Ung. acidi boric*, equal parts. Careful inspection should be made for small vesicles or tender spots, so that these can be promptly treated with carbolic.

The following are illustrative cases :—

Case 1. R. 12th October, 1920. Great pain in ear, meatus swollen and inflamed. Tenderness ant. and below swelling of glands in same positions. Drum slightly reddened, but otherwise normal. Temperature 99°.

13th October. Had no sleep previous night on account of pain, which is now very severe. Meatus more swollen, obscuring drum. Temperature 101°.

14th October. Improved. Small blisters along wall of meatus, yellowish-green in colour. (Culture made).

16th October. Improved. One blister seen. (Culture—*B. pyocyaneus*). Patient thought he was cured and did not return till the 22nd October.

22nd October. Intense pain in ear commenced previous day. Three blisters present close to the drum. (Second Culture made).

23rd October. Pain gone. One blister present. (Culture—*B. pyocyaneus*).

25th October. No symptoms. Raw spot at side of blebs.

29th October. No signs of infection. No further recurrence.

*Treatment* as indicated above.

Case 2. N. 25th June, 1921. Right ear, great pain, one day's duration. Meatus swollen and inflamed. (Culture made).

29th June. Meatus still swollen and painful.

30th June. Pain less, swelling less.

1st July. Still swollen, pain less. (Second sub-culture shows the typical green colour of *B. pyocyaneus*).

2nd July. Great improvement.

4th July. Symptoms nil.

11th July. Pain both ears very acute, one day's duration, meatus in both swollen and inflamed. Yellow blebs present, yellowish-green in colour and very numerous. For the next two days the pain and swelling got steadily worse and patient got little or no sleep. Slight improvement on third and fourth days, but swelling completely blocked meatus.

*Treatment.* The usual routine as described above was continued for five days when patient went to Java and returned five weeks later with the following history :

Treated for one week and symptoms all disappeared, condition apparently cured. One week later relapse occurred and all the symptoms returned. Treated for two weeks, pain disappeared in both ears and right ear cleared up completely, but discharge and swelling still continued in left ear.

23rd August. Right ear normal, except for slight redness of walls of meatus and opacity of drum. Left ear, walls of meatus so swollen as to be in apposition ; pus present at entrance, seen to be oozing from a small sinus, the mouth of which is blocked by a granuloma ; acute tenderness of wall of meatus, but no pain.

*Treatment.* Packing with perchloride proving useless, pure carbolic treatment was started on August 26th. Under this the condition gradually cleared up and by September 5th, all symptoms had disappeared.

15th September. A small bleb appeared, showing how tenaciously the infection clings to the walls of the meatus. This was removed and the site touched with pure carbolic. After this no further trouble was experienced and the condition rapidly cleared up and did not return.

This case lasted in all eleven weeks and illustrates the difficulties met with, as well as clearly showing the efficiency of the carbolic treatment. It also shows that it is not safe to conclude that a case is cured, until all symptoms and signs have been absent for at least two weeks.

## CONCLUSION

In the tropics, infections of the external auditory meatus are extremely common. I believe I have furnished sufficient evidence to show that 'True Tropical Ear' is a clinical entity, and that *B. pyocyaneus* is the cause of the infection.

TABLE I.

DIFFERENTIAL DIAGNOSIS.

| Symptoms | Otomycosis | Other Bacterial | Tropical Ear | Middle Ear disease | Furuncle |
|---|---|---|---|---|---|
| Onset..... | Gradual | Gradual | Very acute | Acute | Acute |
| Pain ..... | Nil | Nil | Very severe | Severe | Very severe |
| Tenderness ... | Nil | Nil | Whole canal involved very severe Pinna. ant. and suf. glands | Nil | Some tenderness glands; severe the site of ab |
| Swelling ..... | Nil | Nil | Whole canal involved; very marked. Pinna. ant. and inf. glands | Nil | In canal at site abscess and ma ant. and inf. gl also |
| Course ..... | Chronic with repeated recurrence | Short | Short with repeated recurrence | | Short, liable to re rence |
| Appearance ... | Blocked with white cheesy wax | Mild inflammation of wall and drum | Yellow vesicle or meatus may be obliterated by apposition of walls | No swelling of meatal walls | Swelling of absc clearly seen |
| Deafness ..... | Depends entirely on mechanical blocking of ear | Slight | Depends on blocking of walls by swelling | Marked | Slight |
| Fever and Malaise ..... | Nil | Nil | Malaise marked fever ranges between 99—101° | Malaise very marked fever to 104° | Malaise marked mild |
| Drum ..... | Very rarely | Unaffected | Slight inflammation only | Bulging of drum and later perforation | Unaffected |
| Etiology ..... | Fungi | Staphylococcus micrococcus Catarrh, *Bal. coli*, etc. | *Bacillus pyocyaneus* | Staphylococcus usually | Staphylococcus us |
| Discharge ..... | Nil | Slight serous | Greenish yellow. Small quantities in later stages, none at first | None at first, and then copious yellow pus | Thick yellow pus abscess breaks |

## EXPLANATION OF PLATE IV.

Fig. 1. Early stage of *Aspergillus glaucus* infection of the ear. Fungus seen growing on the drum and on the walls of the meatus.

Fig. 2. Early stage of *Aspergillus niger* infection of the ear. Fungus seen growing on the walls of the meatus and on the drum.

Fig. 3. Late stage of Aspergillosis. Canal completely blocked with greyish, crinkled material, which is composed of a mass of felt-like fungus and infected wax.

Fig. 4. *Saccharomyces* infection of ear. Walls of meatus unaffected. Drum shows large perforation and two yellow patches.

Fig. 5. *Saccharomyces* infection of ear (later stage). Showing large horse-shoe like perforation of drum.

Fig. 6. *B. diphtheriae* infection of ear. White membrane can be seen growing from the wall of the meatus on to the drum itself.

Fig. 7. 'True Tropical Ear.' 1st stage, showing small, greenish-yellow vesicles on the wall of the meatus. Drum unaffected.

Fig. 8. 'True Tropical Ear.' 2nd stage. Walls of canal red, inflamed and very swollen. Canal almost obliterated by apposition of walls.

Fig. 9. 'True Tropical Ear.' 3rd stage, showing deep-seated abscess, which is discharging pus through a minute sinus. At the mouth of the sinus is seen a small, pouting granuloma.

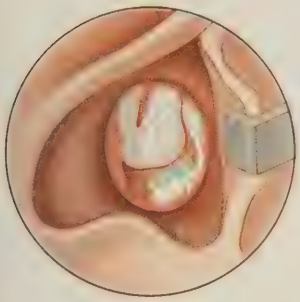
Fig. 10. 'True Tropical Ear.' Canal packed with wool through which the bright green colour of *B. pyocyaneus* shows clearly.

Figs. 11, 12 and 13. Diagrams of Drum in different stages of *Saccharomyces* infection.

Fig. 11. Small perforation and diseased patches devitalized by the Yeast.

Fig. 12. Extended perforation.

Fig. 13. Permanent perforation left after the disease.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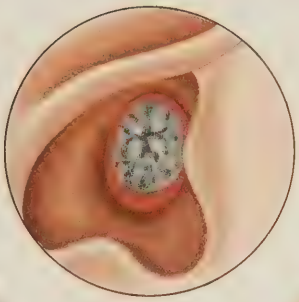
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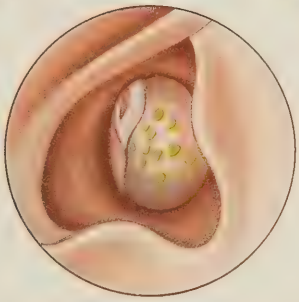
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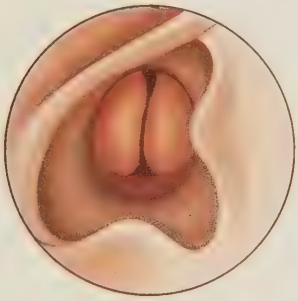
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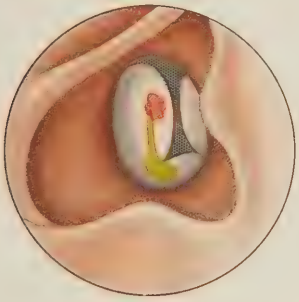
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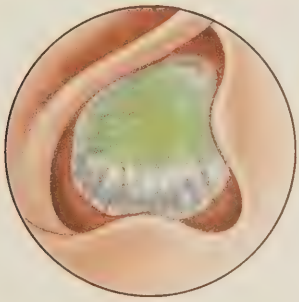
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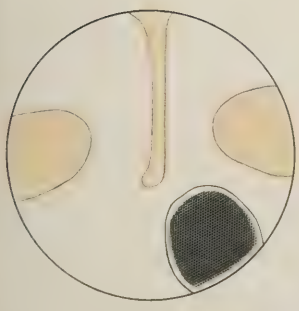
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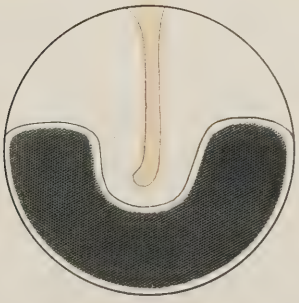
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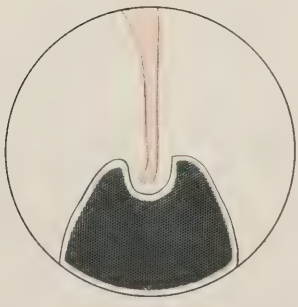
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# ON THE FUNCTION OF THE OESOPHAGEAL DIVERTICULA IN THE ADULT FEMALE MOSQUITO

BY

W. REES WRIGHT, B.Sc.

(*Department of Zoology, University College of North Wales, Bangor*)

(*Received for publication* 18 *February*, 1924)

The Oesophageal Diverticula are three sacs arising from the posterior portion of the oesophagus of the mosquito; they are present in both sexes. One sac, which is many times larger than the two others, lies ventrally in the thorax and the anterior portion of the abdomen, and opens on the ventral surface of the oesophagus at about the level of the first pair of legs; the distance to which it extends backwards in the abdomen varies according to the amount of food present in either the sac or the mid-gut; thus when the sac is full and the mid-gut empty, the former extends to the sixth or seventh segment, while when the mid-gut is full and the sac empty of food, the latter may be seen as a translucent area in the ventral portions of the first two abdominal segments. The two smaller sacs are dorso-lateral in position, lie in the thorax only, and open into the oesophagus at the same level as the large ventral sac. All three sacs contain a gas in the form of numerous bubbles; Hindle (1914) states that this gas is carbon dioxide produced by certain commensal fungi belonging to the *Entomophtoraceae*.

These organs have been described by a number of authors, the most complete and accurate account being that of Nuttall and Shipley (1903). These authors have also investigated the function of the sacs, and regard them as food-reservoirs. Their paper contains valuable discussions of the descriptions of several previous writers, and of various theories as to the function.

Christophers (1901) describes the organs briefly, and states that

'after feeding . . . . blood is very evident in the mid-gut and even in the calyx-like proventriculus, yet in the oesophagus there is no trace . . . . In a fed mosquito a transparent area is generally to be seen in front of the opaque mass of blood in the abdomen. This transparent area is the abdominal portion of the air-containing oesophageal diverticulum.'

Nuttall and Shipley (1903) describe experiments which they carried out to determine the function of the diverticula. These experiments, in which *Culex pipiens* was chiefly used, consisted in feeding the mosquitoes 'with blood-serum and sugar, either alone or together with carmine or neutral red. Sometimes the feedings took place alternately on coloured and uncoloured solution.' The results of these experiments are summarised in the following Table (Table I). Two experiments* (9 and 10) in which the number of insects used is not stated, are not included in the Table, but are given in Notes A and B below.

TABLE I.

Summary of Nuttall and Shipley's Experiments.

Mosquitoes fed on serum-sugar, either plain or coloured.

| | Total | Food sacs | In gut | Remarks |
|---|---|---|---|---|
| Killed at once ... ... ... ... ... | 3 | 3 | 1 | A little in mid-gut of one |
| Killed after 1 hour ... ... ... ... | 1 | 1 | 1 | Most in ventral sac |
| Killed after 24 hours ... ... ... ... | 5 | 5 | 2 | Moderate amount in mid-gut. Note A. |
| Killed after 48 hours ... ... ... ... | 5 | 5 | 1 | Note B. |
| Fed again after 24 hours ... ... ... | 6 | 6 | 6 | Note C. |
| Fed again after 48 hours ... ... ... | 2 | 2 | 2 | Note D. |

NOTE A. 'Experiment 9. Several insects fed on sugar-carmine-serum, killed after 24 hours showed carmine in intestine down to rectum, besides in ventral sac.'

NOTE B. 'Experiment 10. Several insects, treated as in 9, were killed after 48 hours, there being more carmine in intestine and rectum.'

* The numbers of the experiments refer to their order in Nuttall and Shipley's paper.

Note C. 'Experiment 14. Six insects, fed as in the preceding case (sugar-serum tinged with neutral-red) were fed again after 24 hours on clear sugar serum. The result was very striking. The contents of the ventral sac were coloured red, those of the stomach yellow, so that there could be no doubt but that the second meal had been almost entirely taken up by the stomach.'

Note D. In Experiments 4 and 5 the insects were fed as in 14, the interval being 48 hours. In one 'no bubbles in the much distended ventral sac, which contained carmine. Much aggregated carmine in the stomach.' In the other 'large bubbles and carmine in ventral sac. Little carmine in intestine, which contained some clear serum.'

Most writers subsequent to Nuttall and Shipley follow these authors in regarding the diverticula as food reservoirs. Thus, Patton and Cragg (1913) state that they 'are in fact, true food-reservoirs, as was first shown by Nuttall and Shipley. In mosquitoes killed during the act of feeding they are always found full of blood, while a little later, depending on the rate of digestion and, therefore, on the temperature, the blood is almost entirely confined to the mid-gut.' They point out, however, that the conditions of Nuttall and Shipley's experiments were highly artificial. Hindle (1914), also, regards the sacs as food reservoirs, but notes that the question is 'far from settled.'

In the course of some investigations on the Biology of the British Mosquitoes, the present writer found that although insects which had recently fed on blood were fairly common, he never encountered any which had the least trace of blood in the oesophageal diverticula. The species under investigation were *Anopheles maculipennis*, *Theobaldia annulata*, and *Culex pipiens*, and, at a later date, *A. bifurcatus* and *Aedes detritus*. He commenced experiments with *Anopheles maculipennis*, with the results shown in Table II; these experiments, which had to be abandoned shortly after their commencement, consisted in feeding the insects on the human subject. At a later date he was able to resume the investigation; this second series consisted of a repetition of his former work and also of Nuttall and Shipley's experiments using as food an aqueous solution of cane sugar tinged with neutral-red. This second series is summarised in Table III. In connection with the experiments with sugar solution as food, it was observed that the mosquitoes took a far longer time to gorge themselves than they usually take with a blood meal. In this second series of experiments the insects were killed by means

of ether immediately after feeding, and dissected at once. When the gut or sacs were not full of food they were carefully examined under a magnification of at least × 100.

TABLE II

Writer's First Series.

Mosquitoes fed on human subject.

| | Total examined | Condition of Diverticula | | |
|---|---|---|---|---|
| | | Much blood | Trace of blood | No blood |
| Gorged, killed at once ... ... ... ... | 14 | 1 | 3 | 10 |
| Disturbed while feeding, killed immediately ... | 11 | 0 | 3 | 8 |
| Gorged, killed within 15 minutes ... ... ... | 3 | 0 | 1 | 2 |
| Gorged, killed within 60 minutes ... ... ... | 2 | 0 | 1 | 1 |
| Total ... ... ... ... ... ... ... | 30 | 1 | 8 | 21 |

TABLE III.

Writer's Second Series.

Mosquitoes (*Anopheles maculipennis* and a few *Theobaldia annulata*) fed on human subject or on cane sugar—neutral red. All killed immediately after feeding.

| | Total | Sacs | | Gut | Remarks |
|---|---|---|---|---|---|
| | | Much food | Trace of food | | |
| Human subject ... | 18 | ... | 5 | 18 | |
| Sugar—neutral red ... | 23 | 23 | ... | ... | |
| Fresh fruit juice ... | 1 | 1 | ... | ... | A *Theobaldia*, fed on a piece of orange |

When we come to consider the various records, we find considerable discordance. Thus the statements of Christophers and of Patton and Cragg are entirely opposed to each other. The writer's own field observations and part of his experimental work favour Christophers' statements, while Nuttall and Shipley's results, and

those of the writer's experiments with 'artificial food,' strongly support the statements of Patton and Cragg.

It is generally agreed that the original food of the Culicidae was plant juices as it still is to-day in the case of the males of all species and the females of many, and that blood is an 'acquired taste.' If we consider the experimental evidence, we must agree that the experiments in which the food-material used most closely approximated to the primitive food of the insects were those of Nuttall and Shipley, and those of the present writer with sugar solution. In these the Oesophageal Diverticula were undoubtedly functional as food-reservoirs. In the case of blood-meals we are dealing with a more recently acquired character, and consequently must not be surprised to find, as we undoubtedly do, that the organs function irregularly.

In an experiment included in Table III, the writer found that fresh fruit juice was taken up into the ventral sac. In this connection it is interesting to note that Blacklock and Carter (1920) found that females of *Anopheles plumbeus* fed on human blood, 'when it was obvious that they had recently partaken freely of the raisin diet provided.' Presumably, the fruit juice was taken up by the diverticula and the blood by the gut.

The writer would suggest as a provisional hypothesis, that the original function of the Oesophageal Diverticula is that of food-reservoirs, but that this function is largely suspended in the case of blood-sucking females.

It has been suggested by some authors that these sacs, being 'air'-filled, would tend to buoy-up the insect when in flight. It is obvious that they would not function so, unless the density of the gases within was less than that of the external atmosphere, and, if Hindle is correct in his statement that the gas present is carbon dioxide, they would tend rather to weigh the insect down.

## ACKNOWLEDGMENTS

The writer has much pleasure in taking this opportunity of thanking Professor R. Newstead, F.R.S., and Professor Warrington Yorke, M.D. for valuable suggestions as to the presentation of his results.

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# NOTES ON SOME MOSQUITO LARVAE FROM NORTH WALES

BY

W. REES WRIGHT, B.Sc.

(*Department of Zoology, University College, Bangor*)

(*Received for publication* 18 *February*, 1924)

The material which forms the subject of this paper was obtained during the course of some investigations on the breeding-habits of the North Wales Culicidae, the results of which were published in these ANNALS (Rees Wright, 1923).

I. AN UNDESCRIBED LARVAL INSTAR OF *Theobaldia* (*Culicella*) *morsitans* (Theobald). (Fig. 1.)

So far as the writer is aware, the first instar larva of this species has not been described.

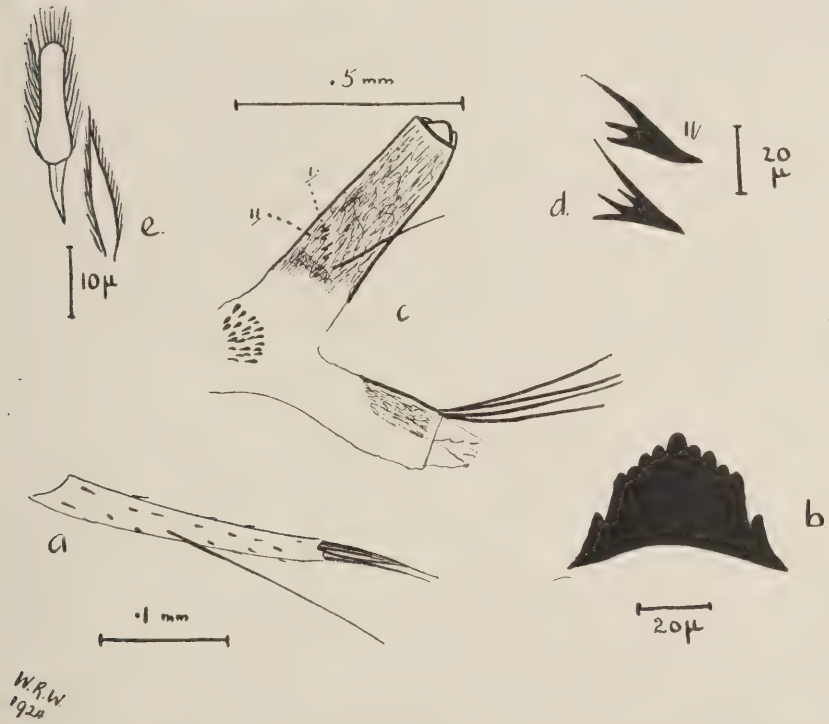


FIG. 1. *Theobaldia morsitans*; 1st Larval Instar. *a*—Antenna; *b*—Mental plate; *c*—Siphon and Anal segment; *d*—Pecten teeth; *e*—Comb-scales.

*Head* about as broad as long, all hairs simple, with an egg-breaking tooth on the dorsal surface. *Antenna* (fig. 1, *a*) shorter than the head, basal tuft a single hair. *Mental plate* (*b*) triangular, with a large median tooth and four smaller teeth on either side, the outermost slightly separated from the rest. No *anal fin*, four long and stout hairs arise from posterior margin of saddle (*c*). Comb-scales (*e*) few, of two kinds similar to those of the adult. *Pecten* oblique, of four teeth in which the main denticle is decidedly larger than the rest, which are not, however, reduced to small projections on it (*d*). *Tufted hair* single. *Siphonal index* about 3.

Glan-rhyd Reservoir, Pentir, near Carnarvon. 21.9.1922.

## II. Differentiation of Larval Instar of *Aedes* (*Ochlerotatus*) *detritus* (Haliday).

Lang (1920) gives a key to the instar of this species; he notes that the characters are not diagnostic 'but should be applied with the reservation that in any one specimen any character may vary beyond the prescribed limits.' The writer obtained some larval pelts of this species which could not belong to the fourth instar, as there were no pupae in the tank in which this species was being bred, but which possessed most of the characters given by Lang for that instar. The writer later obtained the two last larval pelts of a single individual. Table I gives a comparison between the characters found and those given by Lang; Table II gives the extreme range of variation observed in a number of larvae examined. It will be noticed that the characters for the third instar 'overlap' those of the fourth.

Table I.

| | Lang (1920) | | W.R.W. | |
|---|---|---|---|---|
| | III | IV | III | IV |
| Inner post-antennal hair ... ... ... ... ... | 1 | 2 | 2 | 4 |
| Middle post-antennal hair ... ... ... ... ... | 1 | ... | 2 | 2 |
| Outer post-antennal hair ... ... ... ... ... | 3-4 | 4+ | 6 | 10 |
| Pecten teeth ... ... ... ... ... ... ... | 10-14 | 18-30 | 18 | 23 |
| Tufted hair of Siphon ... ... ... ... ... ... | 2-3 | 4-9 | 7 | 1[illegible] |

TABLE II.

| | Instar | |
|---|---|---|
| | III | IV |
| Inner post-antennal hair ... ... ... ... ... ... ... ... | 1-2 | 1-3 |
| Middle post-antennal hair ... ... ... ... ... ... ... ... | 2-5 | 2 |
| Outer post-antennal hair ... ... ... ... ... ... ... ... | 2-6 | 2 |
| Pecten teeth ... ... ... ... ... ... ... ... At least | 18 | ... |
| Tufted hair of siphon ... ... ... ... ... ... ... ... ... | 6-7 | 6-10 |

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# AN *ISOSPORA* OF CIVET CATS

BY

S. ADLER

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown*)

(*Received for publication* 22 *February*, 1924)

PLATE V

An *Isospora* was studied in three heavily infected civet cats, *Viverra civetta*—two young animals in which the infection proved fatal, and one adult which was killed for pathological examination.

The animals passed blood and mucus and a large number of oöcysts in their faeces.

The parasite was studied in scrapings of infected mucosa, in sections and in smears. Smears were prepared in the following way : a portion of infected gut was gently washed in water to remove débris, the mucosa was then removed with a scalpel and gently rubbed on the surface of a slide ; prepared in this way, the relationship between the parasite and the epithelial cells is in many instances hardly disturbed. Some of the smears were fixed in Schaudinn's fluid and stained with iron haematoxylin ; others were fixed in methyl alcohol and stained with Giemsa's stain. If the débris is carefully washed away from the infected mucosa, Giemsa's stain gives good results.

## DESCRIPTION OF THE PARASITE IN THE HOST

Development is confined to the epithelium of the lower half of the small intestine. Although trophozoites penetrate into the tissue they do not undergo further development there ; their nucleus breaks up into granules and they eventually disintegrate, or they are occasionally engulfed in the lymphocytes (fig. 9).

The merozoites vary in size from $4{\cdot}0\mu$ to $9{\cdot}6\mu$ in length by $0{\cdot}8\mu$ to $2{\cdot}4\mu$ in thickness. This large variation depends on the number

of merozoites in the schizonts, on the size of the schizonts, and partly on individual variation. Thus in abnormal schizonts containing four merozoites (fig. 19) these may reach 9·6$\mu$ in length by 2·4$\mu$ in breadth, and on the other hand, in schizonts containing more than eight merozoites (fig. 22) they may be as small as 4$\mu$ in length by 0·8$\mu$ in breadth. In normal schizonts containing eight merozoites which are by far the commonest (figs. 20 and 21) variations in size occur (4·8$\mu$ to 6·2$\mu$ in length by 1$\mu$ to 1·8$\mu$ in breadth). There is no evidence that the large merozoites found in schizonts are differentiated for the sexual cycle. Probably the size of the merozoites in this particular parasite is determined only by the space available for growth inside the schizont and has no sexual significance. The merozoites inside the schizont are usually slightly bent and are pointed at one extremity.

The nucleus of the merozoite is relatively large ; it has a distinct nuclear membrane and contains a large karyosome which may lie in the centre of the nucleus or it may be attached to a portion of the nuclear membrane.

Trophozoites are found inside vacuoles in the infected cell, the pointed end of the parasite being attached to a point in the wall of the vacuole (figs. 1 and 2). Double infection of cells was frequently seen and occasionally three merozoites were found parasitising one cell.

In cases of multiple infection, two trophozoites may be found in one vacuole (fig. 16), or each parasite may lie in its own vacuole (fig. 17).

The trophozoites grow inside their vacuoles and are usually gregariniform in shape. The variations found in the shapes of the trophozoites are shown in figs. 3 to 8.

When the trophozoite has attained a size of 9·6$\mu$ to 11·1$\mu$ in length by 3·2$\mu$ to 3·7$\mu$ in its thickest part, nuclear division takes place. This is accomplished in the following manner :—The karyosome divides by simple fission, and the two daughter karyosomes move to opposite ends of the nucleus ; between them a spindle stretches, on which two plates of chromosomes form ; these move towards the daughter karyosomes and the nuclear membrane then divides by constriction between the two karyosomes, two daughter nuclei being formed. The schizont maintains its gregarine form

till it contains eight nuclei ; it then becomes round, the protoplasm segments and eight merozoites are formed. The schizont may or may not contain a residual body. Abnormal schizonts are found which contain more or less than eight merozoites (figs. 18, 19, 22). Schizonts vary from 10$\mu$ in diameter (containing four merozoites) to 18·4$\mu$. Normal schizonts containing eight merozoites are generally about 16$\mu$ in diameter.

## MICROGAMETOCYTES

As previously stated it was not found possible to distinguish the merozoites destined to become microgametocytes, but after several nuclear divisions have occurred, it is easy to distinguish the microgametocyte from the schizont, for it tends to become round or elliptical when it is only 6$\mu$ in diameter and it then contains about thirty small round nuclei (fig. 30).

When the growing microgametocyte becomes sufficiently large it is possible to see that nuclear division takes place by mitosis (fig. 32). A stage is reached when nuclear division ceases and the microgametocyte then contains a large number of irregular compact nuclei (fig. 33) ; these become round and from each nucleus a small rod of chromatin protrudes (fig. 34). The nucleus then becomes falciform and finally microgametocytes develop (fig. 35).

The completely developed microgametocyte varies from 16$\mu$ to 24·7$\mu$ in diameter and contains one, or more commonly two residual bodies round which the microgametes are grouped. There are usually about two hundred microgametes in one fully developed microgametocyte. The microgamete has two flagella and measures about 10$\mu$ from the tip of one flagella to the other.

## THE MACROGAMETE

The young macrogamete is easily recognisable, for it becomes spherical early in its development, when only 7$\mu$ in diameter. At this stage it is very striking, because of its large round nucleus which takes up half the diameter of the parasite (figs. 23 and 24). The nucleus of the young macrogamete is the same in size as well as structure as that of the mature macrogamete. Deeply staining

granules appear in the protoplasm round the nuclear membrane and as the parasite grows, these granules diminish in number and are disseminated throughout the protoplasm. The nucleus of the host cell becomes attached to and often drawn over the vacuole containing the macrogamete (figs. 26 and 28), but this is not absolutely typical of the macrogamete, for it is also occasionally seen in microgametocytes and schizonts.

The protoplasm of the macrogamete consists of a very fine mesh containing small refractile granules. The mature macrogamete attains almost the same size as the oöcyst. Macrogametes were found to be about thirty times as numerous as microgametocytes.

Fertilisation takes place inside the host cell. As many as nine or ten microgametes were observed inside one macrogamete (fig. 29).

After fertilisation the hard wall of the oöcyst is formed. The protoplasm of the oöcyst contains coarser granules than the macrogamete. In addition to the numerous small refractile granules, the protoplasm contains from one to five larger refractile bodies and these are extruded during the first division of the oöcyst (figs. 36 and 37) forming an oöcystic residue which disappears before development of the oöcyst is completed. The first division takes place inside the invaded epithelial cell or in the gut. Oöcysts containing two sporoblasts are found in the gut with the remains of the nucleus and protoplasm of the invaded cell attached to them. No further development takes place inside the host and the oöcysts containing two sporoblasts are passed in the faeces.

A number of oöcysts are passed with the protoplasm unsegmented but they are probably non-fertilised macrogametes which have become encysted, for they do not appear to develop further outside the body of the host.

## DEVELOPMENT OUTSIDE THE HOST

After twenty-four hours the sporoblasts have a sporocyst round them and become spores, each spore containing two masses of protoplasm and a residual body. The spore measures 12·5$\mu$ to 15·2$\mu$ in length by 8$\mu$ to 11$\mu$ in breadth; the residual body at this stage measures 6$\mu$ by 10$\mu$.

After three days' development is completed, each spore then contains four sporozoites and a residual body ; the sporozoites are sickle-shaped and measure 9·5$\mu$ to 11·2$\mu$ in length by 2·8$\mu$ in their thickest part ; the residual body is more diffused than in the previous stage, so that it is impossible to give definite measurements. After standing for two or three weeks, the residual body in the spore is reduced to a few granules.

The oöcysts vary in size from 19$\mu$ to 27·5$\mu$ in length by 15·2$\mu$ to 24·7$\mu$ in breadth, the commonest size being 22·8$\mu$ by 19$\mu$, and the extremes 15·2$\mu$ by 19$\mu$ and 24·7$\mu$ by 27·5$\mu$ being only two per cent. of the total number measured. They thus approximate in size to, but are slightly larger than *Isospora rivoltae*, Grassi, 1879 (15$\mu$ to 20$\mu$ by 20$\mu$ to 24$\mu$) in English dogs (Wenyon, 1923).

## IDENTITY OF THE PARASITE

The oöcysts are approximately the same size as *Isospora rivoltae*, Grassi, 1879 ; but the *Isospora* of civet cats has certain features which distinguish it from the former.

(1) Its oöcysts are passed containing two sporoblasts whereas in *I. rivoltae* the oöcysts are passed unsegmented.

(2) It was not found possible to infect cats and kittens, and young dogs by feeding with heavily infected material. Two cats, three kittens and two young dogs were used for the experiment, and they failed to become infected within an observation period of twenty-eight days.

The *Isospora* of civet cats is therefore probably not the same species as *Isospora rivoltae* and I propose the name *Isospora viverrae*, n.sp., for the parasite.

## PATHOLOGY OF THE INFECTION

On section, the infected parts of the small intestine showed that in the basal parts of the villi few cells were infected, whereas the distal parts were heavily infected. A similar observation was made by Wenyon in the case of *Isospora felis*, Wenyon, 1923. In many villi the distal part was entirely denuded of the epithelium. The sub-epithelial tissue in infected areas was markedly hyperaemic.

In spite of the heavy infection and the changes of the sub-epithelial tissues no ulceration was observed. In this connection it is interesting to note that during 1922 and 1923 about thirty specimens of *Viverra civetta* were examined in the Sir A. L. Jones Laboratory, in Freetown, and in no case was ulceration of the small intestine noted. It seems probable, therefore, that regeneration takes place from the epithelium of the basal and comparatively unaffected portions of the villi.

An interesting fact was observed, namely, that in heavily infected parts of the small intestine islands were found which on serial section appear to be free from infection. It is hardly likely that the cells of these portions surrounded by heavily infected areas were not subjected to invasion by merozoites. A more feasible explanation is, that these islands of healthy mucosa had been infected and the villi denuded of epithelium distally ; regeneration from the healthy epithelium situated at the base of the villi then took place and the regenerated areas became immune to further infection, i.e., we are dealing with a case of local and acquired immunity to a protozoon.

In the two young animals, numerous schizonts as well as sexual forms were found, whereas in the adult animal schizonts were comparatively few and were only found after prolonged search ; it is probable, therefore, that the adult animal was on the eve of a spontaneous cure in spite of a heavy infection.

## SUMMARY AND CONCLUSIONS

An *Isospora* was studied in three heavily infected civet cats.

The asexual and sexual cycles within the host are described.

Oöcysts containing two sporoblasts were found in the intestine and in fresh faeces.

The oöcysts measured 19$\mu$ to 27·5$\mu$ in length by 15·2$\mu$ to 24·7$\mu$ in breadth.

The development of the oöcysts outside the body of the host is described.

Cats and dogs fed on heavily infected material failed to become infected within an observation period of twenty-eight days.

The name *Isospora viverrae*, n.sp., is proposed for the *Isospora* of civet cats.

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## EXPLANATION OF PLATE V

| | |
|---|---|
| Figs. 1, 2. | Trophozoite inside vacuole. |
| Figs. 3-8. | Various forms of trophozoites. |
| Fig. 9. | Trophozoite inside lymphocyte. |
| Figs. 10-12. | Trophozoites showing nuclear division. |
| Figs. 13-15. | Schizogony. |
| Fig. 16. | A vacuole containing two parasites, a merozoite and one young schizont. |
| Fig. 17. | One cell with two vacuoles each containing a parasite. |
| Fig. 18. | Schizont with four small merozoites. |
| Fig. 19. | Schizont with four large merozoites and a residual body. Note large size of merozoites and residual body. |
| Figs. 20, 21. | Schizonts with eight merozoites. |
| Fig. 22. | Schizont with 13 merozoites showing. |
| Figs. 23-28. | Showing development of macrogamete. Note in 26, 27 and 28, application of nucleus of host cell to wall of vacuole. Note in young macrogametes the large size of the nucleus. |
| Fig. 29. | Macrogamete invaded by a number of microgametes. |
| Fig. 30. | Young microgametocyte. |
| Fig. 31. | Later stage of microgametocyte. Note relationship of the nucleus of host cell to vacuole. |
| Fig. 32. | Later stage of microgametocyte showing mitotic figures and irregular masses of chromatin. |
| Fig. 33. | Still later stage of microgametocyte showing solid masses of chromatin. |
| Fig. 34. | Still later stage of microgametocyte. From each mass of chromatin a small process protrudes. |
| Fig. 35. | Still later stage showing falciform nuclei and one residual body. |
| Fig. 36. | Oöcyst as found in mucosa. |
| Fig. 37. | Oöcyst as found in mucosa and passed in fresh faeces. |
| Fig. 38. | Fully developed oöcyst. (Drawn from material three months' old, hence the residual bodies are small) × 1250. |

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# *ORNITHODORUS MOUBATA*, Murray, IN RELATION TO RELAPSING FEVER IN THE GOLD COAST

BY

A. INGRAM, W.A.M.S.

(*Received for publication 27 February*, 1924)

The carrier of African tick fever *Ornithodorus moubata*, which Brumpt (1922) definitely states does not convey *Sp. recurrentis*, appears to be absent from the Gold Coast and consequently cannot have played any part in the recent outbreak of relapsing fever at Accra. An opportunity, however, was given of testing the efficiency of this species as a carrier of the strain of spironemata causing the epidemic in the Gold Coast, when a consignment of *O. moubata*, hailing originally from Rhodesia, was received in September at Accra from the Liverpool School of Tropical Medicine, thanks to the courtesy of Dr. J. W. Scott Macfie.

The ticks were kept unfed in the incubator for ten days after their arrival, so that they might recover from an apparent state of lethargy induced probably by the conditions encountered on their journey. On the 14th September eighteen of the ticks were placed on a pouched rat, *Cricetomys gambianus*, Waterhouse, which had been inoculated with blood from a case of relapsing fever and was showing a heavy infection with spironemata. Almost all the ticks employed were recovered in an engorged condition the next morning and were despatched at once to Liverpool, where they arrived safely, but, according to information received from Dr. Macfie, failed to convey infection to two rabbits and a monkey upon which they fed. The remaining ticks of this consignment, sixteen in number, were divided into two lots, which were placed upon two different pouched rats that had been inoculated on the 7th September from a case of relapsing fever and which showed numerous spironemata in their blood. On the 15th September, the morning after they had been

placed upon the rats these ticks were returned to the incubator, where they were kept until the 2nd October. On this date, all the surviving ticks which had fed on the two infected rats on the 14th September, were placed upon a clean pouched rat. Next morning, however, as only one or two of them seemed to have fed they were again returned to the incubator and the feeding was repeated upon the same rat on the 16th and 30th October by which time the number of ticks had been reduced to four owing to the propensity of the rat to devour them. Thick films of blood from this rat were examined daily from the beginning of the feeding experiments till the 22nd November but spironemata were never found in them and preparations made from the body fluid of the four surviving ticks, which had presumably fed on the rat on one of the occasions on which it was exposed to their attack, failed to show spironemata.

On the 30th November eleven specimens of *O. moubata* from a second consignment, kindly forwarded by Dr. Macfie, were placed on a pouched rat which was showing spironemata abundantly in its blood. Only eight of these ticks, of which five were engorged, were recovered on the following morning and were placed in the incubator. On the 4th December, fourteen fresh ticks were placed on the same rat, which was still showing a few spironemata in its blood ; all of these ticks, eight being engorged, were recovered on the morning following and placed in the incubator.

Seven of the ticks, which had been placed on the infected rat on the 30th November, were placed on a clean pouched rat on the 13th December. Unfortunately, only two of them could be found the next morning and neither appeared to have fed. The blood of this rat never showed spironemata in thick films which were examined daily till the end of December.

On the 20th December the ticks which had been placed on the infected rat on the 4th December were placed on another clean pouched rat ; eleven of them were recovered on the following morning and were returned to the incubator, several being in an engorged condition. As this last rat experimented with did not show spironemata in thick films of its blood examined daily up to the 4th January, it was on that date again exposed to the bites of fifteen *O. moubata* which had fed previously on infected *C. gambianus*, yet no spironemata were found after this second exposure to the

attacks of ticks, which had every opportunity of becoming infected, although the blood of the rat was examined daily until the 31st January.

It would appear from these feeding experiments that *Ornithodorus moubata*, Murray, is not an efficient carrier of the spironemata causing the recent epidemic of relapsing fever in the Gold Coast.

## REFERENCES

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SELWYN-CLARKE, P. S., LE FANU, G. H., and INGRAM, A. Relapsing Fever in the Gold Coast. *Ann. Trop. Med. & Parasitol.*, Vol. XVII, pp. 389-426.

# THE RELATIONSHIP BETWEEN THE *ASCARIDS* OF MAN, PIG AND CHIMPANZEE

BY

H. THORNTON

(*From the Parasitology Department of the Liverpool School of Tropical Medicine*)

(*Received for publication* 1 *March*, 1924)

The material available for examination was :—

(1) Numerous specimens of *Ascaris lumbricoides* obtained by Dr. J. W. S. Macfie from Asylum patients at Accra.

(2) Numerous specimens of *Ascaris suilla* from pigs killed in the Liverpool Abattoir.

(3) A few *Ascarids* obtained from a chimpanzee (*Anthropopithecus* sp.).

Attempts were made to establish any difference especially in regard to the lips, shape of the pulp and number and position of the cephalic papillae. The tails of the male worms were compared

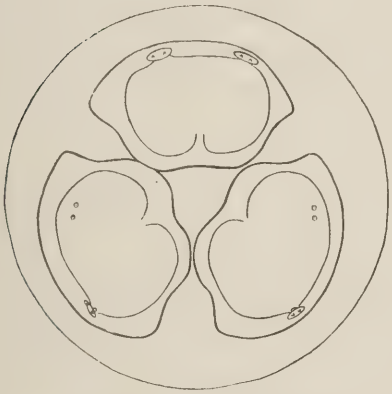

FIG. 1. *Ascaris lumbricoides.* Head, anterior view.

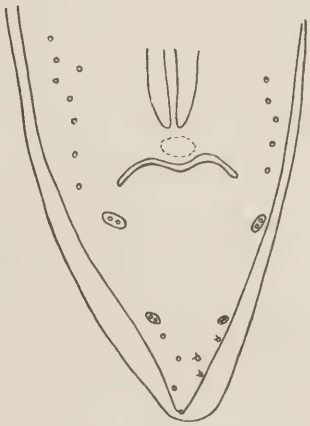

FIG. 2. *Ascaris lumbricoides.* Post-extremity of male, ventral view.

as regards the mode of termination and the number and distribution of the papillae on the ventral surface. The spicules in the male were examined and in the female the position of the genital opening.

On the dorsal lip of the head are two large double papillae; on each of the sub-ventral lips is one large double papillae lying towards the ventral aspect and, more laterally, two small papillae lying close together. This arrangement exists in worms from all three sources. The pulp of the lips appeared to show no difference in the various worms.

The tail of the male terminates in a small mammillate projection. It varies somewhat in shape, but such variations occur not only in worms from different sources, but in worms from the same host. The number and position of the postanal papillae in the male is constant. There are seven pairs, the two anterior being double, and of these the one nearer the cloaca is always the larger. The remaining three pairs are single and arranged on each side in the form of a triangle with the apex directed inwards. Immediately in front of the cloaca is a large cushion-like structure. The preanal papillae are irregular, but in worms from all three sources there are at least 50 pairs. The spicules are broad, flattened dorso-ventrally and non-alate, no differences being found in the worms from the various sources.

Morphologically, then, the *Ascaris* of the pig and of the chimpanzee cannot be distinguished from the *Ascaris* of man, and should, therefore, be designated *Ascaris lumbricoides*, Linn (1758).

These observations agree with those of Baylis and Daubney (1922), who examined and compared the *Ascaris* from man, orang-utan, Indian wild pig and various squirrels.

## REFERENCE

Baylis, H. A., and Daubney, R. (1922). Report on the Parasitic Nematodes in the Collection of the Zoological Survey of India. *Mem. Ind. Mus.*, Vol. VII, No. 4, p. 263.

# *ASCARIS LUMBRICOIDES* CAUSING FATAL LESIONS IN A CHIMPANZEE

BY

A. W. N. PILLERS

(*Received for publication 7 March,* 1924)

On June 6, 1922, I conducted a post mortem examination on a young female chimpanzee, which had died soon after importation into this country. There was no satisfactory clinical history. After being listless for a few days, she had been found dead one morning; the autopsy was made about twenty-four hours after death. The body was moderately well nourished. The face, ears and those areas of the skin not densely covered with hair, were distinctly icteric. A careful examination of all the cavities and their contained organs showed that gross abnormalities were confined to the small intestine and liver.

The duodenal mucous membrane was discoloured by numerous dark particles—presenting an appearance similar to that of a moderately anthracosed lung. The remainder of the intestinal mucous membrane was slightly inflamed. In the lumen were sixty-three large round worms ranging in length from 6·5 cms. in the case of males to 15·75 cms. in females. These on examination proved to be *Ascaris lumbricoides* (*vide* Thornton, 1924). The liver weighed about 6 ozs. The right or main lobe contained three abscesses, each about the size of an ordinary marble and in each of them was a degenerated worm measuring about 9 cms. in length. In the left lobe was a partly decomposed female worm about 13 cms. in length, bent on itself like a hair pin, each limb running a tortuous course in what appeared to be dilated bile ducts. At the junction of the hepatic and cystic ducts was a considerable dilatation which continued down the ductus choledochus for a short distance. In the sac so formed were six partly decomposed worms which varied in length from 5 to 7 cms. Two were bent on themselves, but the

others were not. There were thus four worms in the liver and six in the duct, making with the sixty-three in the small intestine a total of seventy-three.

The result of the autopsy leads one to conclude that the animal died as a result of the infestation with *Ascaris lumbricoides*, and that death was due more particularly to those worms which had gained the liver, apparently by way of the bile duct.

## REFERENCE

THORNTON, H. (1924). The Relationship between the *Ascarids* of Man, Pig and Chimpanzee. *Ann. Trop. Med. & Parasitol.*, Vol. XVIII, pp. 97-98.

# THE ACTION OF THE SALIVARY SECRETION OF MOSQUITOS AND OF *GLOSSINA TACHINOIDES* ON HUMAN BLOOD

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication* 7 *March*, 1924)

Whilst conducting experiments with malaria sporozoites from the salivary glands of *Anopheles maculipennis*, we were much impressed by the extraordinary degree of agglutination of the erythrocytes which immediately occurred on mixing an emulsion of salivary gland with citrated human blood. On looking through the literature, we were surprised to find that apparently the only record of this phenomenon is that of Cornwall and Patton (1914), who state that the salivary secretion of *A. rossi* and *A. jamesi* produced strong and immediate agglutination of the red corpuscles of human blood and prevented the blood from clotting. Experiments were undertaken to investigate this subject and to contrast the action on human blood of the salivary secretion of female *A. maculipennis* with that of female *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides*.

In all experiments, the emulsion of salivary glands was obtained by breaking up as finely as possible with needles the entire salivary glands from a single insect in 0·035 c.c. of physiological saline solution, and the citrated blood was obtained by adding three volumes of human blood to one volume of a 1 per cent. solution of sodium citrate in physiological saline solution ; one volume of the salivary gland emulsion was then mixed with two volumes of the citrated blood solution and the mixture drawn up into a capillary tube of rather wide bore.

In the case of *A. maculipennis* complete and immediate agglutination of the erythrocytes was observed, whereas in the case of *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides*, respectively, no trace of agglutination of the erythrocytes was seen, even after standing an hour at either 37° C. or laboratory temperature. The experiments, which were repeated several times, were performed with blood from two normal individuals and gave constant results.

Observations were next made in order to obtain some idea of the concentration of the agglutinin in the salivary gland emulsion of *A. maculipennis*. For this purpose the original emulsion containing the complete salivary glands of one mosquito in 0·035 cc. of saline solution was diluted 4 times, 8 times, 16 times, 32 times, 64 times and 128 times, respectively, and as in the previous experiments one volume of the various dilutions mixed with two volumes of citrated blood. Complete and immediate agglutination of the red cells was observed up to a dilution of 32-fold; with the 64-fold dilution there was a considerable immediate agglutination which became complete after standing a little time, while in the case of the 128-fold dilution the amount of agglutination observed was but slight. These observations show clearly that haemagglutinin is present in the salivary glands of *A. maculipennis* in very great concentration.

The agglutination of red cells due to the salivary secretion of *A. maculipennis* takes place about equally well at 37° C. as at laboratory temperature of about 15° C.

Heating the emulsion of salivary gland of *A. maculipennis* for half an hour at 56° C. completely destroys the agglutinin. Further experiments showed that exposure to a temperature of 50° C. for half an hour also completely destroyed the agglutinin, whilst heating to 45° C. for a similar period, produced some slight diminution in its intensity. The haemagglutinin in the salivary secretion of *A. maculipennis* is hence very thermolabile and is destroyed at comparatively low temperatures. When a mixture of emulsion of salivary glands of *A. maculipennis* and blood—the red cells of which are thus completely agglutinated—is heated at 60° C. for two minutes, it was found, on discharging the blood on a glass slide, that the agglutinated masses of red corpuscles had completely dissociated

and no longer re-agglutinated when the temperature fell to 15° C. Heating the mixture to 50° C. for fifteen minutes partially destroyed the agglutinated masses, whilst heating at the same temperature for thirty minutes caused complete disappearance of the agglutination.

The haemagglutinin present in the salivary glands of *A. maculipennis* differs, therefore, in certain respects from the autoagglutinin present in the blood of certain normal animals, and more markedly in the blood of these animals when suffering from certain infections, e.g., trypanosomiasis: firstly, in that the salivary gland agglutinin acts about equally well at 37° C. as at low temperatures, and secondly, in that it is very thermolabile, being destroyed at 50° C.

If the salivary gland emulsion of *A. maculipennis* is allowed to dry at either 15° C. or 37° C. and the residue re-dissolved in a volume of fluid equal to the original volume of the emulsion, it is found that on adding two volumes of citrated blood no agglutination takes place: the agglutinin is thus destroyed by dessication.

Adopting the same technique, it was found that the salivary secretion of *A. maculipennis* agglutinated strongly, but not quite so intensely as in the case of man, the red blood corpuscles of the donkey, rabbit and dog, but had no action on those of the mouse, guinea-pig and monkey (*Cercopithecus* sp.). The salivary secretion of *Stegomyia fasciata* had no agglutinating action on the red corpuscles of any of these animals.

Emulsions of the stomach and of the ventral oesophageal diverticulum of *A. maculipennis* failed to produce any agglutination of human erythrocytes.

No evidence of haemolysis was observed in any of the experiments performed with the salivary gland emulsion of *A. maculipennis*, or any of the other mosquitos, or of *Glossina tachinoides*.

Experiments were finally undertaken with *A. maculipennis*, *Stegomyia fasciata* and *Glossina tachinoides* to determine whether the salivary gland emulsion exerted any inhibitory action on the coagulation of human blood. The salivary gland emulsions were made as described above, the complete glands of one insect being emulsified in 0·035 cc. of physiological saline. One volume of the emulsion was then mixed with two volumes of blood as it escaped from the freshly-punctured finger and after mixing thoroughly,

drawn up into a capillary tube of rather wide bore and placed in a water bath at 37° C. The control tubes containing one volume of physiological saline and two volumes of blood were invariably found to have clotted after five minutes, so that when the contents of the tube were blown out on to a glass slide, a long worm-like red clot was extruded : this red cylindrical mass could not be disintegrated by stirring with a needle or by warming to 60° C., as would be the case if the red cells were merely agglutinated. The salivary secretion of *Stegomyia fasciata* had no effect on the coagulation, the blood having clotted as in the controls within five minutes ; the salivary gland emulsion of *A. maculipennis* merely delayed the coagulation of the blood, no clot being observed after five minutes, but a distinct clot was found after fifteen minutes. In the case of *Glossina tachinoides*, however, the anticoagulating power of the salivary gland emulsion was much more pronounced, no coagulation of the blood being observed after standing at 37° C. for thirty minutes and little, if any, even after twelve hours.

In view of the above observations one is led to enquire what happens to the blood which is drawn into the mosquito stomach on feeding. It was found that the blood in the stomach of *A. maculipennis* two hours after feeding on a human being, exhibited complete agglutination of the erythrocytes, but was unclotted, whereas that in the stomach of *Stegomyia fasciata* a similar time after feeding, exhibited no agglutination of the erythrocytes, but was quite definitely clotted. This suggests that when the mosquito feeds, salivary secretion is first poured out into the wound and then partly withdrawn with the blood into the stomach. The fact that sporozoites were found free amongst the blood in the stomach of an infected *A. maculipennis* two hours after feeding, is additional evidence in support of this; as the mosquito had been infected over five weeks before the meal in question, the sporozoites found amongst the blood in the stomach could not have been contaminations from oöcysts in the stomach—and none were found in the stomach on dissection—but must apparently have had their origin in salivary secretion which, after being discharged into the wound, had been withdrawn with the blood into the stomach. Furthermore, a little blood is occasionally to be found in the ventral oesophageal diverticulum of an *A. maculipennis* when examined an hour or two after feeding

and sporozoites have been found amongst the blood in this situation, a couple of hours after feeding.

We can offer no explanation of the function of the haemagglutinin or anticoagulin in the salivary secretion of *A. maculipennis*. So far both these bodies have been shewn to be present in the salivary secretion of all the *Anopheles* examined, viz., *A. rossi*, *A. jamesi* and *A. maculipennis*, but both are absent in that of *Stegomyia fasciata*, whilst in *Glossina tachinoides* the salivary secretion contains a powerful anticoagulin, but no agglutinin.

## SUMMARY

1. An emulsion of the salivary glands of *A. maculipennis* powerfully agglutinates the erythrocytes of human blood and, less strongly, those of the donkey, rabbit and dog ; it has no agglutinating action on the red corpuscles of the mouse, guinea-pig or *Cercopithecus* sp. Emulsions of the stomach and of the ventral oesophageal diverticulum exerted no agglutinating action.

2. The haemagglutinin is thermolabile being destroyed by heating to 60° C. for two minutes or to 50° C. for thirty minutes, and the agglutinated red cell masses dissociate completely under these conditions.

3. Similar salivary gland emulsions of *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides* do not agglutinate human red cells, nor does the salivary gland emulsion of *Stegomyia fasciata* agglutinate the red cells of any of the animals mentioned above.

4. No haemolysin was detected in the salivary gland emulsion of any of these insects.

5. The salivary gland emulsion of *Stegomyia fasciata* exerted no anticoagulating action on human blood, whilst in the case of *A. maculipennis* and of *Glossina tachinoides*, the anticoagulating action was quite definite.

6. The blood in the stomach of *A. maculipennis* two hours after feeding on a human being was found to exhibit complete agglutination of the erythrocytes, but to be unclotted, whilst that in the stomach of *S. fasciata*, a similar period after feeding on man, exhibited no agglutination, but was coagulated.

7. This indicates that the salivary secretion is in part withdrawn into the stomach with the blood when the mosquito feeds; further evidence in support of this is the fact that sporozoites were found intermingled with the blood in the stomach and ventral oesophageal diverticulum of infected *A. maculipennis* examined shortly after feeding.

## REFERENCE

Cornwall, J. W., and Patton, W. S. (1914). Some Observations on the Salivary Secretions of the Commoner Insects and Ticks. *Ind. Journ. Med. Res.* Vol. II, p. 569.

## ADDENDUM

Since writing the above, we have had an opportunity of examining the reactions of the salivary secretion of *A. bifurcatus* and find that it neither causes agglutination of human erythrocytes nor does it delay coagulation of human blood. In these respects, therefore, *A. bifurcatus* differs strikingly from *A. maculipennis*, *A. rossi*, and *A. jamesi*.

# ON THE CLASSIFICATION OF THE CESTODE GENUS *MONIEZIA* (BLANCHARD 1891)

BY

G. THEILER

(*From the Parasitology Department of the Liverpool School of Tropical Medicine*)

(*Received for publication* 25 *March*, 1924)

The investigation, of which the present article is the outcome, was suggested to me by Professor Warrington Yorke, who kindly put all the material of the Liverpool School of Tropical Medicine at my disposal. I wish here to tender my thanks to him, and also to Mr. Southwell for allowing me to make use of his prepared slides, as well as for his friendly assistance and advice.

The museum collection contained material from cattle, sheep, and goats from Port Said, Malta, Accra, Bâle, and different localities in England. As most of this material was fragmentary and as there could thus be no certainty that the contents of any one bottle originally belonged to one worm only, it was decided to procure fresh material. This was obtained from the Liverpool Abattoirs during the months of May to September, 1923, and only entire worms were kept. All these were preserved and cleared in the same way, in order that so far as possible the differences due to preservation, etc., might be avoided.

It has recently become customary, when examining large cestodes, to select small portions from various positions of the strobila and to identify the species on the data so obtained (Sauter). This rough and ready method is very convenient and may be sufficient in old and established genera, where the differences between the various species are well-defined. But in the genus *Moniezia* it is apt to lead to confusion. The examination of entire worms—a most laborious

proceeding—enables one not only to see the variations present in different specimens, but also variations which may be present in any one worm. In this way, I have been able to show that several differences which were looked upon as specific may, and actually do occur in a single individual.

## HISTORICAL

Blanchard in 1891 created the genus *Moniezia* with the following diagnosis :—

'Corps lancéolé en avant, anneaux serrés, beaucoup plus larges que longs, avec deux pores sexués, opposés,'

and included in it the following eleven species :—

*M. alba*, Perroncito 1878
*M. benedeni*, Moniez 1874
*M. denticulata*, Rudolphi 1804
*M. expansa*, Rudolphi 1810
*M. festiva*, Rudolphi 1819
*M. goezei*, Baird 1853
*M. leuckarti*, Riehm 1881
*M. marmotae*, Fröhlich 1862
*M. neumanni*, Moniez 1891
*M. nullicollis*, Moniez 1891
*M. pectinata*, Göze 1782

Moniez (1891) included *T. ovilla*, Riv. 1878, and *Thysanosoma actinioides*, Diesing 1834, in this new genus.

Stiles and Hassall (1893) emended Blanchard's definition as follows :—

'Head without hooks ; segments generally broader than long and longer than thick ; end segments shewing a tendency to become longer and narrower. Two full sets of genital organs, with two uteri and two lateral pores in each segment. On the right side the vagina is ventral, cirrus dorsal ; on the left side vagina dorsal, cirrus ventral. Dorsal canal lies dorso-median of the ventral canal. Genital canals cross the longitudinal canals and nerves dorsally. Interproglottidal glands generally present. Calcareous bodies absent from parenchyma. Eggs with well-developed pyriform body.'

Type species *M. expansa* (Rudolphi 1810) Blanchard 1891.

Stiles and Hassall included in this genus eight species which fell into three groups :—

(*a*) *Planissima* group, characterized by the linear arrangement of the interproglottidal glands.

*M. planissima*
*M. benedeni*
*M. neumanni*

(*b*) *Expansa* group, characterized by the saccular arrangement of the interproglottidal glands.

*M. expansa*
*M. oblongiceps*
*M. trigonophora*

(*c*) *Denticulata* group, in which the interproglottidal glands are absent.

*M. denticulata*
*M. alba*

As *Moniezia* they include five of Blanchard's original species and further describe three new species. Later, Stiles classifies as *Cittotaenia* the double-pored leporine forms, which differ from *Moniezia* in the following particulars :—

'Vagina ventral to the cirrus-pouch on both sides of the segment; interproglottidal glands absent; generally one, but in some cases two, simple transverse tubular uteri in each segment; uterus generally possesses simple, proximal and distal diverticula. Eggs with a well-developed pyriform body, the horns of which are long, generally filamentous and cross each other. Type-species *Cittotaenia latissima*, Riehm 1881 = *Cittotaenia denticulata* (Rudolphi 1804), Stiles and Hassall 1896.'

In this genus Stiles includes four of Blanchard's original *Moniezia*, namely :—

*M. denticulata* = *Cittotaenia denticulata*
*M. goezei* = *Cittotaenia denticulata*
*M. marmotae* = *Cittotaenia marmotae*
*M. pectinata* = *Cittotaenia pectinata*

*M. nullicollis*. Stiles does not consider this to be a well-established species, as knowledge of its anatomical details is lacking.

*M. festiva*. He mentions this species as being recorded as a *Moniezia* by Blanchard. Nybelin (1917) has since re-examined Rudolphi's original specimen and has made it the type species of a new genus *Hepatotaenia*, closely allied to the genus *Cittotaenia*.

Fuhrmann (1902) placed the double-pored avian cestodes—the genus *Paronia* of Diamare 1900—into the genus *Moniezia*. They

were left here until 1918, when Fuhrmann accepted the genus *Paronia*. This genus differs from *Moniezia* in the arrangement of its genital ducts; in the structure and origin of its uteri, and in the absence of a pyriform apparatus in the egg.

Since the revision of the adult cestodes of cattle, sheep and allied animals by Stiles and Hassall, several new *Moniezia* have been described, namely :—

*M. rugosa* (Diesing 1850), Lühe 1895, in *Ateles hypoxanthus*.
*M. amphibia*, von Linstow 1901, in *Hippopotamus amphibius*.
*M. minima*, Marotel 1912
*M. triangularis*, Marotel 1913
*M. conjungens*, Sauter 1917
*M. latifrons*, Sauter 1917
*M. crassicollis*, Sauter 1917
*M. parva*, Sauter 1917
*M. pellucida*, Blei 1920
*M. translucida*, Jenkins 1923
*M. chappuisi*, Baer 1923

The question of the validity of these species will be considered below.

## GENERAL STRUCTURE OF THE GENUS *MONIEZIA*

The segments are usually very much broader than long (fig. 9), though worms may be met with in which the breadth may only be three times the length (figs. 6 and 7). These variations are attributable to the state of contraction or relaxation of the different sets of muscles, and to a certain degree they are specific. Thus, as a rule, *M. planissima* has a broader strobila than *M. expansa*. The actual shape of the segment, or the length of the complete strobila, cannot be considered as of specific value. I have been able to stretch a fragment of live worm to at least five times its original length, without damaging it. Also most worms undergo an appreciable amount of contraction when placed in weak formalin, and further contraction may take place when clearing in clove oil.

The length of the strobila also depends on the age of the worm; for instance, an individual which has but newly established itself will be short, and mature segments will be reached within a short

distance of the scolex. I have seen specimens of the *expansa* group, 30-50 cms. in length, in which the last segments were gravid. In older worms the strobila may be anything up to three metres long. In this case the difference between any two succeeding proglottides is not appreciable, and maturity will only be arrived at at a considerable distance from the head. I have seen several fragments, measuring 70 cms.-1 metre in length, in which all the proglottides were at the same stage of development.

The amount of overlapping is also dependent on the state of contraction of the various muscles. One individual may show broad proglottides with overlapping edges, where the longitudinal muscles have contracted, whereas a few inches further back the longitudinal muscles may be relaxed and the circular muscle-layer be contracted, giving the segment a longer, narrower appearance, with hardly any overlapping of the posterior edges.

The question of shape and size has here been gone into in detail, because these points have been taken to be of specific value.

All the authors are agreed that the shape of the head and the length of the neck are liable to show a great deal of variation. On the whole, the scolex and neck of the *expansa* group can be said to be long and slender, whereas in the *planissima* group they are squat and broad, even when in a fairly relaxed state. I have drawn several heads (figs. 1-5), all taken from typical *Moniezia expansa*, showing the shapes they may assume, according to the sets of muscles contracted. Fig. 3 resembles the head of *M. expansa* as drawn by Stiles and Hassall (1893), whereas fig. 5 corresponds with their drawing of *M. oblongiceps*.

The nervous, excretory and muscular systems are essentially the same throughout the genus (see Tower 1900, Zschokke 1888) so need not be dealt with here.

### The Expansa Group

In this group the cells of the interproglottidal glands are arranged around blind sacs. To this group have been ascribed *M. expansa*, *M. oblongiceps*, *M. trigonophora*, *M. minima*.

The interproglottidal glands are usually well-developed and stain readily. In material, however, which had been preserved for several years, I occasionally had great difficulty in making them take the stain at all. They were likewise often very difficult to see

Fig. 1.

Fig. 3.

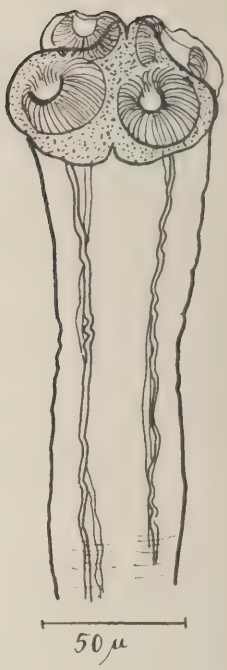


Fig. 4.

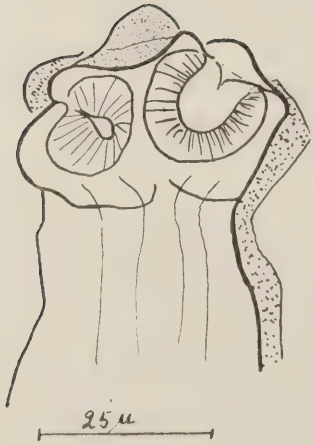


Fig. 2.

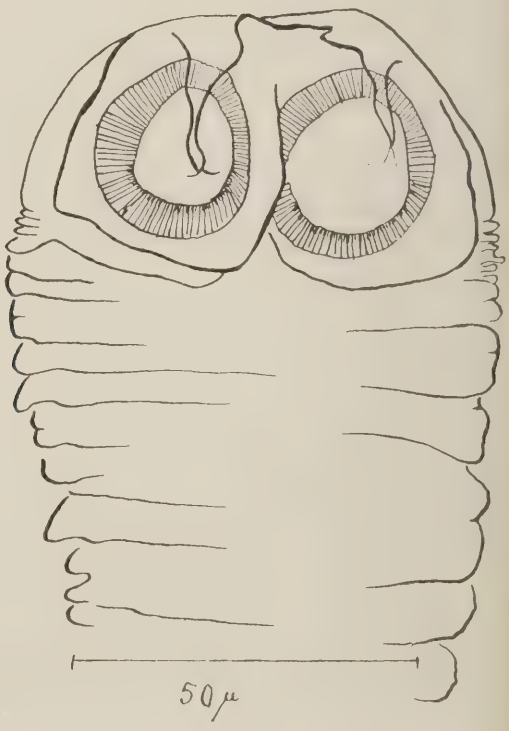


Fig. 5.

Figs. 1-5. Heads of typical specimens of *M. expansa*.

in strongly-contracted specimens. Nevertheless, a diligent search of the whole strobila never failed to reveal their presence. In relaxed specimens, where the glands were easily visible, they were seen to increase in number, in proportion to the width of the segment. In gravid segments they may be obscured by the number of eggs present (fig. 12). Among the numerous specimens examined, I found one worm, apparently normal in all respects, and in a relaxed condition, which showed some of its segments devoid of all traces of glands. After every seven or eight ordinary segments, one or two of these abnormal ones were interposed.

Stiles and Hassall give the smallest number of glands as twenty-five for *M. expansa.* I have seen many specimens of typical *M. expansa,* collected in this country, in which the number was much less. As few as seven glands may often be met with, and some mature segments may never have more than about fifteen. Thus the number of glands present and their prominence cannot be taken as of specific value.

The arrangement of the female genitalia is essentially the same in all the species described.

The arrangement of the testes, however, shows a certain amount of variation. In *M. trigonophora,* the testes are roughly arranged in two right-angled triangles; the base of the triangle is nearly parallel to the posterior edge of the segment; the perpendicular is parallel with the lateral margin; the hypothenuse runs from the antero-lateral portion to the posterior edge near the median line. These triangles never meet in the mid-line. In *M. expansa,* according to Stiles and Hassall, numerous testes are present, and they occupy the median line as well as the rest of the median field. According to Zschokke, the testes of *T. expansa* are arranged in two triangles, one each side of the segment, as is the case in *M. trigonophora.* Stiles and Hassall further remark that by far the greater number of segments of *M. expansa* examined by them did not present this relation, although in a few segments of this species, which were collected at Paris, the triangular arrangement of the testes is very distinct. In Rudolphi's specimens the testes are not visible.

My experience is that the testes in *M. expansa* may be roughly arranged in two broad triangles which usually meet in the mid-line (fig. 6), or in a continuous band thinning slightly towards the

FIG. 6. 1mm.

FIG. 7. 1mm

FIGS. 6 and 7. Segments of *M. expansa* showing irregular arrangement of testicular fields.

mid-line (fig. 7), or they may be as numerous in the median field as elsewhere (fig. 8). All three types may be found in one individual, or only one type may be present. On the whole, I am inclined to think that this question of arrangement also depends on the state of contraction of the segment. It can be readily conceived that Fig. 7 is Fig. 6 with the circular muscles slightly contracted, and that in Fig. 8 we have still further contraction of the circular muscles. As Child rightly points out, the arrangement of the reproductive organs may vary widely in correlation with the variation in form of the proglottid. There is also a variation in the number of testes in different segments of one strobila.

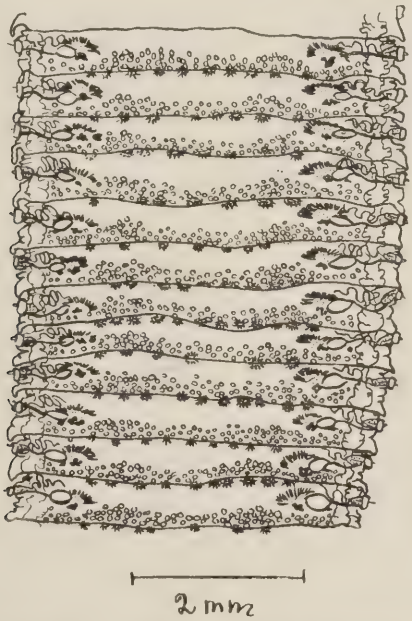


Fig. 8. Contracted segments of *M. expansa*, showing testes arranged in a continuous band.

*M. oblongiceps* agrees with *M. expansa* in all respects, except that the testes are smaller and less numerous. The description of this species is based on two entire strobila and one fragment. As both specimens are under one metre long and are gravid, it seems that we are dealing with a parasite newly established in the gut. The description, when regarded from this point of view, agrees with that of a young *M. expansa*. Thus *M. oblongiceps* must be considered as a synonym of *M. expansa*.

The characteristic features of *M. minima* are given as follows: gravid segments square (anneaux ovigères carrés); testes arranged in a continuous band. As pointed out above, the shape of the segments depends entirely on the amount of contraction present (figs. 6-8), and the arrangement of the testes shows a considerable amount of variation. Hence *M. minima* must be regarded as a variation of *M. expansa*.

The *expansa* group thus contains but two species: *M. expansa*, in which the testes are roughly arranged in a band stretching to the excretory vessels on either side, and *M. trigonophora*, in which the testes are arranged in two triangular fields that do not meet in the mid-line.

It is possible that forms intermediate between *M. trigonophora*, with its two triangles not meeting in the mid-line, and the variation of *M. expansa* as drawn in Fig. 6, may still be found. However, until further variations have been demonstrated, it is preferable to consider the two as separate species.

### The Planissima Group

In this group the cells of the interproglottidal glands are arranged in a line at the juncture of the segments. Ten species have been described which have this linear arrangement of the interproglottidal glands, namely:—

*M. benedeni* (Moniez 1879), R. Blanchard 1891
*M. neumanni*, Moniez 1891
*M. planissima*, Stiles and Hassall 1892
*M. triangularis*, Marotel 1913
*M. conjungens*, Sauter 1917
*M. latifrons*, Sauter 1917
*M. crassicollis*, Sauter 1917
*M. parva*, Sauter 1917
*M. pellucida*, Blei 1920
*M. translucida*, Jenkins 1923

The glands in this group have been described as short and ill-defined (*M. triangularis*), or large and distinct (*M. planissima*), or first linear and later arranged round three blind sacs (*M. conjungens*). These differences are more apparent than real, as the glands in any one strobila may vary a great deal in length. The dorsal gland may

be considerably longer than the ventral, or *vice versa*, or both may be approximately equal in length (fig. 9). Again, the linear gland may be broken up into smaller parts (fig. 9). I have never observed these cut-off portions to be arranged around blind sacs as described

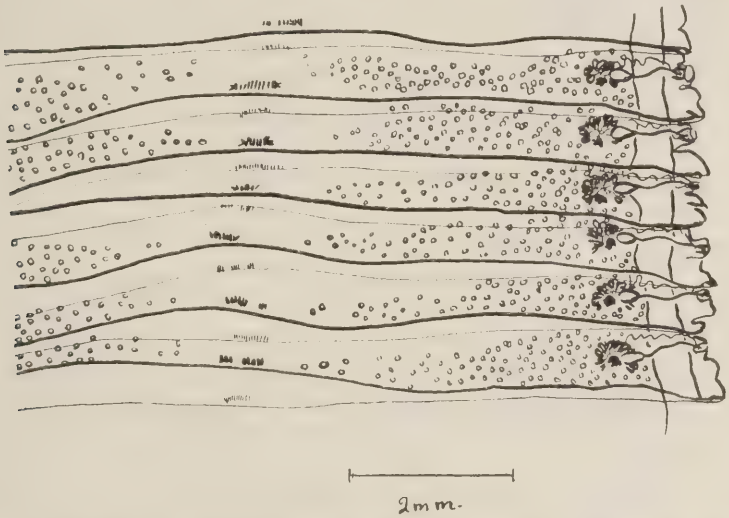


FIG. 9. Segments of *M. planissima*, showing testes arranged in two triangles; glands entire or broken.

by Sauter; on the contrary, the glandular cells always persist in their linear arrangement. In all the relaxed specimens examined, glands were discernible in each proglottid.

The testes in this group, as in the *expansa* group, show a great deal of variation in their arrangement. In the usual typical form they are arranged in two irregular triangles which may or may not meet in the mid-line (figs. 10 and 11), the two triangles may be some distance apart (fig. 9), or the testes may merge into one another and form a continuous band, stretching from the one longitudinal excretory vessel to the other (fig. 12). These differences may appear indiscriminately in young immature segments, in old segments, or in adjoining segments.

I could establish no constant relationship between the average width of the segment, the arrangement of the testes and the length of the gland or its tendency to break up into smaller portions. On the contrary, all the evidence goes to prove that each of these factors varied independently of the others. The different combina-

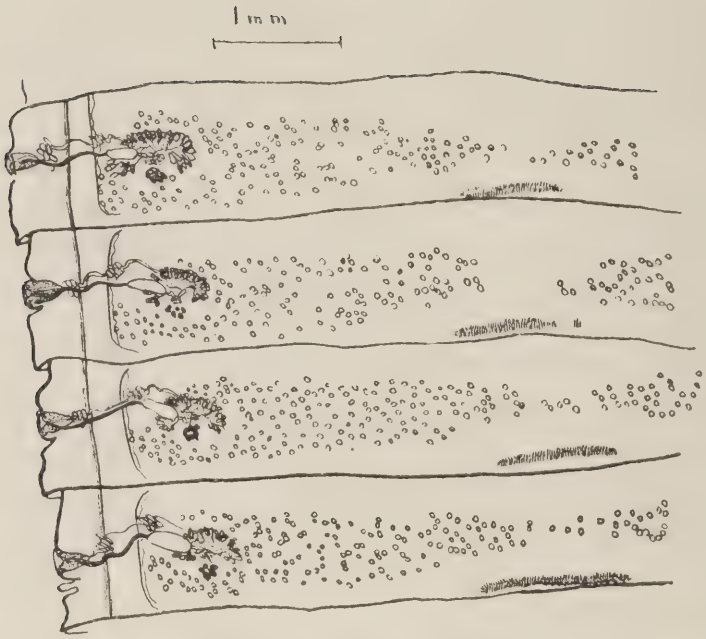


FIG. 10. *M. planissima*, showing irregular arrangement of testicular fields; glands entire or broken.

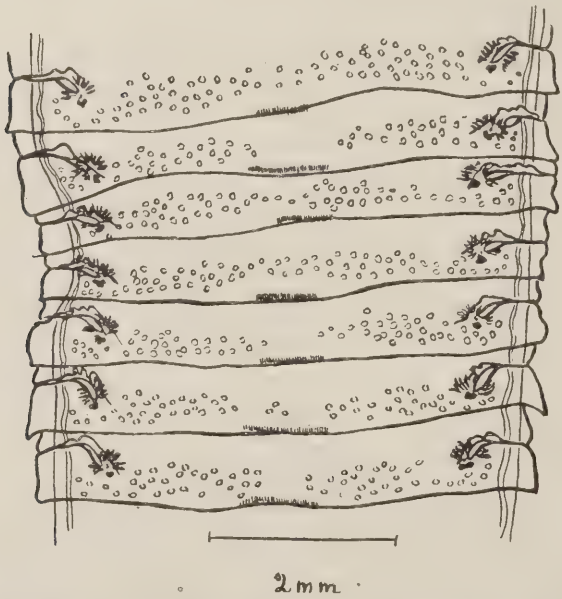


FIG. 11. *M. planissima*, showing irregular arrangement of testicular fields. In this strobila this irregular arrangement persists until maturity is reached. In later segments the glands are broken up.

tions possible would give the various species described; as, for example: small linear gland, testes in two triangles = *M. triangularis*; small linear gland, testes in a continuous band = *M. neumanni*; large linear gland, testes sometimes in two triangles and sometimes a continuous band = *M. planissima*; large gland, testes in a continuous band = *M. translucida*. Unfortunately, however, these arrangements cannot be considered as constituting separate species, as any number of intermediate forms are possible; they are rather to be looked upon as variations of *M. planissima*, with the other described species as links in the chain. In his account of

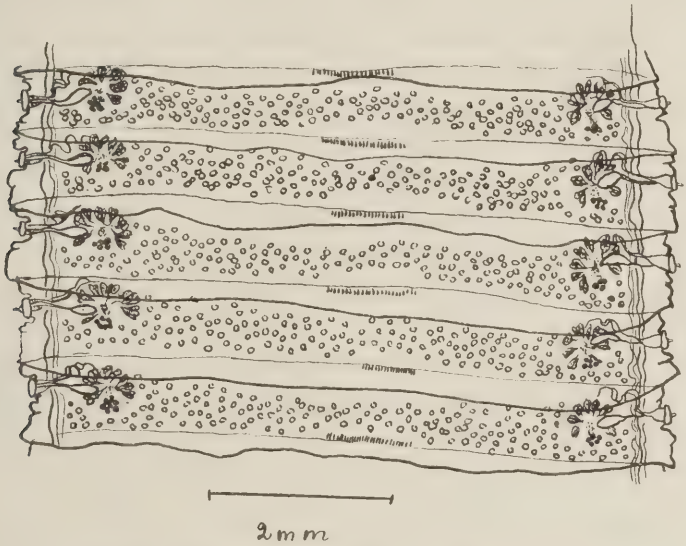


FIG. 12. *M. planissima*, showing testes arranged in a continuous band; glands entire or broken.

*M. conjungens*, Sauter (1917) describes the interproglottidal gland as first linear and later arranged around three blind sacs. As I have never seen this arrangement, but as the linear gland is often broken up into several smaller parts, I assume that this is what Sauter saw, although he draws them arranged round blind sacs.

The *planissima* group thus contains but one species, which will have to be designated *M. benedeni*, in which the head is comparatively large, the testes may be arranged in two triangular fields, or in a continuous band; and the proglottidal gland is linear, and of varying length.

### The Alba Group

This group is based on the fact that its members have no interproglottidal gland. In the material put at my disposal by Mr. Southwell were several bottles labelled *M. alba*, the determination in each case having been based on the examination of a fragment taken from about the middle of the worm. On examination of the whole strobila, however, interproglottidal glands were invariably found under high magnifications. Most of these specimens were either much contracted and distorted, or had been kept in alcohol for a considerable length of time, so that they did not stain well.

With only the above evidence to support the theory, it would be unjustifiable to deny that there may be *Moniezia* without any glands at all, especially in view of the fact that I have seen a well-preserved specimen in which the glands were definitely absent in some of the segments. This absence of the interproglottidal glands may either be due to degeneration of the worm, or possibly to maceration of the material, or to some other cause. Until more work has been done on the presence and function of these glands, *M. alba* must be accepted as a valid species.

Baer (1923) described a new species, *M. chappuisi*, from an antelope, in which no glands were discernible. His description is based on a contracted specimen, and as it shows no essential differences from the description given for *M. alba*, it must be considered as synonymous with that species.

### Species Inquirendae

#### *Moniezia rugosa* (Diesing 1850), Lühe 1895

Lühe 1895 re-examined Diesing's original specimen of *T. rugosa* from the monkey, *Ateles hypoxanthus*. He describes it as a typical *Moniezia*, but does not mention any glands. His oldest segments were not mature enough to enable him to describe the eggs.

If this is a true *Moniezia*, it will fall into the *Alba* group. The question of its validity can only be settled by further work on the parasites of *Ateles*.

#### *Moniezia amphibia*, v. Linstow 1901

This worm is reported from *Hippopotamus amphibius*, where it was present in large numbers. From the description and figures

given, it is difficult to decide whether it is a *Moniezia* without interproglottidal glands, or any other double-pored anoplocephalid. In the drawing of the egg, the horns of the pyriform apparatus may be interpreted as possessing a disc or as having their tips bent over.

Until the original material has been re-examined, this species will have to be considered as a *Moniezia*, of the *Alba* group.

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# *TRYPANOSOMA EVANSI* AND *ORNITHODORUS CROSSI*

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication* 25 *March*, 1924)

A series of experiments recently published by Cross (1921-23) suggests that *Trypanosoma evansi* of the camel in India is biologically transmitted by the tick *Ornithodorus crossi*, Brumpt, 1921. Cross fed *Ornithodorus crossi* on dogs heavily infected with *T. evansi* and found that when the ticks were, after various intervals, subsequently fed on clean animals, some of the latter became infected. In his earlier paper (1922) he states that 'These ticks transmitted the disease to healthy rabbits sixty-seven, eighty-three and one hundred and one days after feeding on an infected-surra animal, but they were not infective after one minute to forty-six days. This would apparently show that there is a life cycle of the trypanosome within the tick'; and in a later paper (1923) that 'These ticks were found capable of spreading the disease to a healthy animal seventeen days and one month after feeding on an infected animal.'

On 10 January, 1923, we received from Captain Cross about two hundred *Ornithodorus crossi*, which had been on 4 December, 1922, fed on a dog with numerous *T. evansi* in its blood. These ticks were divided into four batches, the first three being fed on rabbits and the fourth on a guinea-pig. Between January and June, 1922, the ticks were given frequent opportunities of feeding, but none of the rabbits nor the guinea-pig became infected. It should be mentioned that from the time of their arrival in England, viz., 10 January, 1923, the ticks were kept in an incubator at about 20° C.

It occurred to us, however, that a possible explanation of the failure of these ticks to infect was the fact that immediately after

the infecting feed they were subject to relatively low temperatures during their journey from India to England in the month of December. In order to examine this question Captain Cross sent us a second supply of about two hundred *Ornithodorus crossi*, which were fed on a heavily infected dog on 23 July, 1923. These ticks, which were received by us on 24 August, 1923, were divided into two batches, each of which was allowed to feed on a rabbit on 24 August, and at frequent intervals subsequently up to date: no infection resulted.

We have no explanation to offer for these negative results, but the subject is one of such considerable practical importance as to demand re-investigation.

In order to ascertain what happens to trypanosomes taken into the stomach of the ticks, we fed on 27 June, 1923, a number of the first lot sent by Captain Cross on a rabbit in the blood of which were numerous *T. rhodesiense*. Two months later, two of these ticks were dissected and were found to contain blood in which large numbers of motionless trypanosomes were not only easily recognisable, but were in a state of remarkable preservation. A suspension in normal saline solution of the stomach contents of one of these ticks was inoculated into a mouse intraperitoneally, but failed to produce an infection. In a second, similar, experiment, a tick was dissected on the day after it had fed on a rabbit infected with *T. rhodesiense*. The trypanosomes found in this tick were also motionless and appeared to be dead, and when inoculated intraperitoneally into a mouse failed to infect it.

This observation does not, of course, touch the question of the possible biological development of *T. evansi* in the ticks, but is of interest in showing that trypanosomes which die rapidly in the stomach of the tick remain there for prolonged periods in an excellent state of preservation.

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# MISCELLANEA

## DURATION OF INFECTION IN MALARIA

(*a*) *Quartan.*

P. left India, 1910. No fever and no quinine taken while in England, until November, 1915, when quartan parasites were found in the blood.

(*b*) *Simple tertian.*

N. 9.10.1921, simple tertian parasites.

February, 1922, left West Indies where it is possible a new infection was contracted.

February, 1922, to January, 1924. Sailing between England and North America only.

21.1.24 simple tertian parasites.

J. W. W. Stephens.

## STRONGYLIDAE FROM EDINBURGH HORSES

Since the beginning of October I have had the opportunity to collect and partly examine the Strongylidae found in horses in the Scottish capital. As some of the species present have not as yet been recorded as occurring in either England or Scotland, I thought it interesting enough merely to give a list of the species I have been able to identify so far, but hope to deal more fully with this subject at a later date.

The following are present in the collected material :—*Strongylus vulgaris, S. equinus, S. edentatus, Gyalocephalus capitatis, *G. equi, *Craterostomum mucronatum, Trichonema coronatum, T. bicoronatum, T. calicatum, T. poculatum, T. insigne, T. goldi, T. euproctus, T. nassatum, *T. nassatum* var. parvum, **T. tridentatum, T. mettami, *T. minutum, *T. longibursatum, *T. pseudo-catinatum, *T. pateratum, *T. labratum, *T. labiatum, *T. radiatum.*

P. L. le Roux, B.Sc., M.R.C.V.S.

Zoology Department,
University of Edinburgh,
17.12.23.

* To my knowledge these nematodes have not as yet been reported to occur in British horses, except a foal which furnished Yorke and Macfie with examples of *Gyalocephalus equi* and *Trichonema pseudo-catinatum* after it had been in the same field as American horses which died shortly after importation in 1918.

## ANCYLOSTOMES IN A LEOPARD

Six specimens of *A. braziliense*, Gomez da Faria, 1910, three males and three females, were recovered from a young leopard in Sierra Leone. The females varied from 7·7 to 9·4 mm. in size, the males from 6 to 7 mm.

Eleven specimens of *A. caninum* (Erc., 1859) Hall, 1913, were also recovered, six males and five females. The females varied in size from 7·3 to 8·5 mm., the males from 5·6 to 7·5 mm.

S. ADLER.

## OCCURRENCE OF *ASCARIS LUMBRICOIDES* IN A DRAINAGE TUBE

Dr. Webb Anderson of China has presented to the Liverpool School of Tropical Medicine a specimen of *Ascaris lumbricoides* found in a drainage tube from an appendix abscess. The patient, a Chinese woman, was operated on successfully for appendicitis. Progress was normal for three or four days, after which drainage ceased, and was accompanied by a rise of temperature. On removing the drainage tube it was found that a specimen of *A. lumbricoides* had entered the tube, thus preventing drainage. A photograph of the worm *in situ* is shown below.

T. SOUTHWELL.

Natural size *M. Brown*

## *OPHIOTAENIA MARENZELLERI*

A specimen of *Ophiotaenia marenzelleri*, La Rue 1911, (= *Ichthyotaenia marenzelleri*, Barrois 1898) was found in the intestine of *Causus rhombeatus* in Freetown, Sierra Leone.

This cestode has previously been recorded by Barrois from *Ancistrodon piscivorous* in the southern United States of America.

T. Southwell and S. Adler.

## *ZSCHOKKEELLA GUINEENSIS*

*Zschokkeella guineensis* (Graham, 1908) was found in the intestine of a ground pig, *Thrinomys swinderianus*, in Freetown, Sierra Leone.

This parasite has previously been recorded from *Cricetomys gambianum* in Accra.

T. Southwell and S. Adler.

## TETRARHYNCID LARVA

A Tetrarhyncid larva was found in the liver of a fish *Diodon hystrix* caught near Freetown, Sierra Leone.

T. Southwell and S. Adler.

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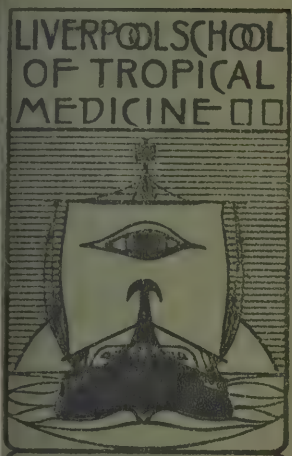


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# ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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## ERRATA

### Vol. XVIII, No. 1

| | |
|---|---|
| P. 34. | For Plate II *read* Plate III. |
| | For Plate III *read* Plate II. |
| P. 35. | Line 6, delete the words Fig. 13. |
| P. 114. | For 50$\mu$ and 25$\mu$ *read* 500$\mu$ and 250$\mu$. |
| P. 129. | For Tetrarhyncid *read* Tetrarhynchid. |

# A NOTE ON *PLASMODIUM AGAMAE* (WENYON, 1908)

BY

S. ADLER.

(*Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication*, 28 *February*, 1924)

Wenyon (1908) working in the Sudan, described a protozoon from the red cells of a lizard *Agama colonorum*, which had the following characters :

The smallest forms found in the red cells were oval or pear-shaped and contained no pigment, but larger immature forms containing brown pigment were also found ; the sexual forms, microgametocytes and macrogametocytes, were sausage-shaped. The affected red cells were not altered in shape or size, but the nucleus was in some instances slightly displaced by the sexual forms of the parasites.

Schizonts were very few and only the early stages were found in the blood ; in no case was a schizont found which had segmented into merozoites. Wenyon, therefore, assumed that multiplication took place in the internal organs after the manner of asexual multiplication, as described by Arago for *Halteridium* and he concluded that the parasite he found in *Agama colonorum* had affinities to *Halteridium* and suggested the name *Haemoproteus agamae*, Wenyon (1908).

It must be pointed out, however, that the animal from which Wenyon described his parasite was not heavily infected.

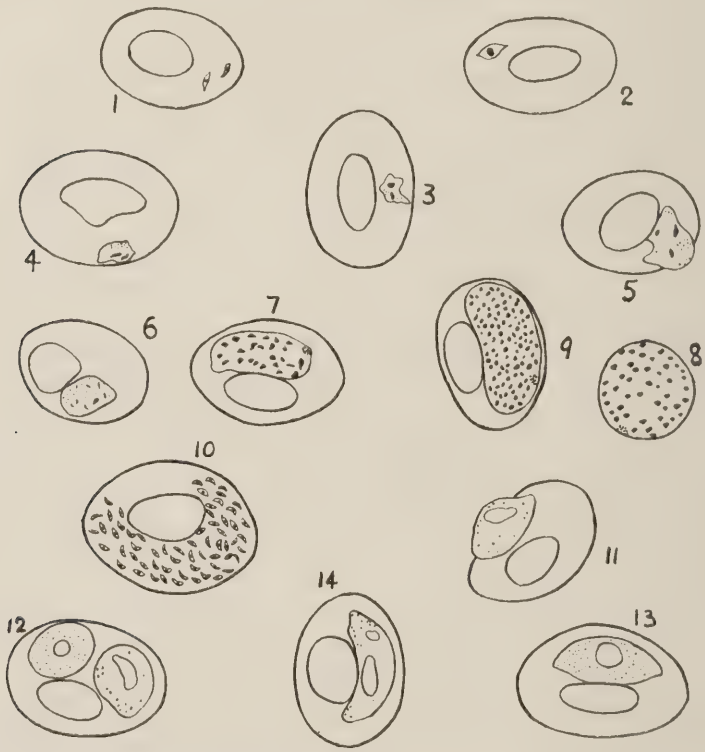
Todd and Wolbach (1912) reported and figured a pigmented haemocytozoon from *Agama colonorum*, which closely resembled the parasite described by Wenyon, but the small forms of the parasite were not found.

Macfie (1914) working in Nigeria, found in the blood of three lizards pigmented parasites which he considered to belong to the same species as those described by Wenyon.

The author found in Sierra Leone a specimen of *Agama colonorum* heavily infected with a pigmented haemocytozoon which appears to be the same species as that described by Wenyon under the name *Haemoproteus agamae.*

Asexual forms, trophozoites and schizonts, were numerous, and it was found that schizogony takes place in the red cells in the peripheral blood. The parasite, therefore, has no affinites to *Halteridium*, but is in fact, a *Plasmodium* and its name should be *Plasmodium agamae* (Wenyon, 1908).

The parasite was studied in smears, and the following points were noted. The smallest forms found in red cells are minute, and




| | |
|---|---|
| Figs. 1 and 2. | Young trophozoites in a red cell. |
| Figs. 3, 4 and 5. | Amoeboid forms with several masses of chromatin. |
| Figs. 6—9. | Further stages of schizogony. |
| Fig. 10. | Schizont which has segmented into merozoites. |
| Fig. 11. | Young microgametocyte, partly outside the red cell. |
| Fig. 12. | A red cell containing a young microgametocyte and a young macrogametocyte. |
| Fig. 13. | A macrogametocyte. |
| Fig. 14. | A microgametocyte. × 1200. |

spindle-shaped, and contain no pigment (figs. 1 and 2). Slightly larger forms are found, very irregular in shape, containing fine granules of pigment and two or more nuclear masses (figs. 3 to 5). Such forms are commonly found in the red cells, but they may also occur either free or partially in the red cells. It seems, therefore, that trophozoites and young schizonts can travel from one red cell to another, unless the free or partially free forms are due to an artifact. Multiple infection of the red cell is common. As the schizont increases in size the nucleus of the red cell is displaced.

The pigment tends to be extruded from the schizont during its growth (fig. 7), and the mature schizont may contain no pigment at all ; the mature schizont contains about seventy merozoites.

The young sexual forms are round or ovoid and are found both free and in the red cells. A few instances were noted in which a young sexual form was found partly inside and partly outside a red cell (fig. 11).

The mature sexual forms are crescentic in shape and seldom displace the nucleus of the red cell (figs. 13-14).

The macrogametocyte stains deep blue with Giemsa, its pigment granules are very fine and are uniformly scattered throughout the protoplasm. The microgametocyte stains pale blue with Giemsa, its pigment is coarser than that of the macrogametocyte and tends to be distributed round the rim of the parasite. The protoplasm of the microgametocyte was in several instances observed to be vacuolated (fig. 14).

# RAT-FLEAS IN FREETOWN, SIERRA LEONE

BY

B. BLACKLOCK

AND

M. G. THOMPSON

(*From Sir Alfred Lewis Jones Research Laboratory, Freetown*)

(*Received for publication* 30 *March*, 1924)

Hirst's (1923) work has attracted attention to the importance which must be attached to the relative proportion of various species of *Xenopsylla* on rats in places which appear liable to plague epidemics. He concluded from his observations that *X. astia* was a much less efficient transmitter of plague than is *X. cheopis*.

In Freetown a hundred rats sent by the Sanitary Department were examined during the months of January and February, 1923: the rats came from various parts of the town; the numbers were:—

| | |
|---|---|
| Black rats ... ... ... ... | 62 |
| Brown rats ... ... ... | 38 |

All the fleas removed from the rats were collected, a total number of 657. Of these 654 belonged to the genus *Xenopsylla* and 3 to the genus *Ctenocephalus*. The 654 *Xenopsylla* comprised 419 fleas of the species *X. brasiliensis* Baker (1904), and 235 of *X. cheopis* Rothschild (1903). The *Ctenocephali* belonged to the species *C. canis* Dugès. In the table are shown the numbers and sex of the rats harbouring *Xenopsylla* and the species recovered from them.

No *X. astia* were found on these rats; Evans (1922), however, records this species among rat fleas sent to her from the Gold Coast.

*Xenopsylla brasiliensis*, Baker (1904) was originally described from Sierra Leone, but its capacity for transmitting plague bacilli has not

so far been worked out. Whether it exhibits that relative inability which Hirst attributes to *X. astia* in Ceylon is unknown.

It is of interest to note that Newstead and Evans (1921), who examined 469 black rats from ships in Liverpool (59 of these rats being obtained from ships coming from various West African Ports), do not record any *X. brasiliensis* from the 469 rats examined, whereas they found 489 *X. cheopis*. Again, Balfour (1922) did not report any *X. brasiliensis* on 34 black and 444 brown rats obtained mostly in London, whereas he records *X. cheopis* on 5·9 per cent. of the black rats and 3·6 per cent. of the brown rats. Hirst (1923) states that *X. brasiliensis* is found on the rats of West Africa, South America and the uplands of Peninsula India.

Table I.

| | Total | Number infested | *X. brasiliensis* | *X. cheopis* |
|---|---|---|---|---|
| Black Rats ♂ ... ... ... | 21 | 19 | 68 | 36 |
| ,, ♀ ... ... ... | 41 | 30 | 74 | 75 |
| Brown Rats ♂ ... ... ... | 17 | 15 | 185 | 87 |
| ,, ♀ ... ... ... | 21 | 19 | 92 | 37 |
| | — | — | 419 | 235 |

Cragg (1920) states that *X. brasiliensis* is not common in India and cites Poona, Mangalore, Bombay City, and Ootacamund as the only places from which it had then been received by him.

Owing to the smallness of the numbers of West African ship-rats examined here and in Liverpool, it is not yet possible to say whether *X. brasiliensis* is capable of remaining alive during transport on ships to England.

Whether *X. brasiliensis* is a plague-transmitting flea or not, it appears probable from our figures that there is a sufficiently large percentage of *X. cheopis* present on rats in Freetown to carry plague effectually in epidemic form, should this disease be imported.

The number of rats in Freetown is large, and, owing to the extensive area which is at present capable of providing natural

shelter for them, it is evident that vast numbers would survive any ordinary efforts at reduction.

The city of Freetown possesses many quarters in which the native population is overcrowded and it contains, also, a large floating population. All these factors are of importance in the case of plague epidemics. The chief protection of this port, in the past, against the introduction of plague from other coastal regions has probably been the absence of a deep-water wharf.

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# A CONTRIBUTION TO THE KNOWLEDGE OF THE TREMATODE PARASITES OF THE FOOD MAMMALS OF RANGOON

BY

G. D. BHALERAO, M.Sc.

(*Biological Department, University of Rangoon*)

(*Received for publication* 4 *April*, 1924)

PLATES VI-VIII

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## INTRODUCTION

The present paper is the outcome of an examination of the Trematodes found in the bullocks, cows, buffaloes, goats, sheep, and pigs slaughtered at the municipal abattoirs, Rangoon. *Fasciola gigantica*, Cobbold, 1858, was found only in bullocks, cows, and buffaloes, always in the liver, and in large numbers. Thus the infection in buffaloes is sometimes 10 per cent., and in bullocks and cows 4 per cent. and 2 per cent. respectively. *Paramphistomum cervi* (Zeder, 1790) and *Eurytrema dajii*, n.sp. sometimes accompany *F. gigantica* in the bile ducts of *Bos indicus*. In goats and sheep the infection is very small, amounting in some months to only 2 per cent. This infection, as reported by the municipal officials, is due to *Fasciola hepatica* L. 1758. No opportunity presented itself of examining these parasites, all the goats and sheep proving to be non-infected whenever a visit was paid to the slaughter-house. Two varieties of pigs are killed in Rangoon, one from Burma, the

other imported from the east coast of India, the latter of these being specially rich in Trematodes. Two species occur in their intestines, *Fasciolopsis füllebornii*, Rodenwaldt, 1909, and *Testifrondosa cristata*, n.sp., the latter being rare.

It is my pleasant duty here to thank Professor F. J. Meggitt who helped me at almost every stage of my work and Mr. G. E. Gates of Judson College, who kindly allowed me the use of his preparations of *Testifrondosa cristata*.

### *PARAMPHISTOMUM CERVI* (Zeda, 1790)

Numerous specimens of this species were found in the bile-ducts of *Bos indicus*, together with many *Fasciola gigantica*. The material confirmed in all respects the description already given by Maplestone (1923).

### *FASCIOLA GIGANTICA*, Cobbold, 1858

Innumerable specimens of this species agreeing in all points with the ' Rangoon specimens ' of Jackson (1921) were found blocking the bile-ducts of *Bos indicus* and *Bos bubalis*. *F. aegyptica*, Looss 1896, appears to be identical with it.

### *F. HEPATICA*, L. 1758

Specimens of this species are reported to have been found in the bile-ducts of sheep and goats, but no cases have been observed by me and no definite records exist.

### *FASCIOLOPSIS FÜLLEBORNII*, Rodenwaldt, 1909 (Plate VI)

Many specimens of this species were found in the intestines of pigs (*Sus cristatus*, Wag., 1900) killed at the slaughter-house, Kemmendine, Rangoon. Apparently they are not firmly attached to the walls of the intestine, as numbers of them quickly emerge when it is washed. The specimens ranged from very immature forms (5·5 mm. by 2·2 mm.) to large ones (55 mm. by 16 mm.), both measured living and fully extended in luke-warm water. These specimens undergo very great contraction when placed in a fixing fluid and become very thick. The immature forms are

rather elliptical, with their greatest width at the level of the anterior testis and with no indication of a cephalic cone at their anterior ends.

The mature forms appear to be tongue-shaped when completely extended and at their anterior end a short cephalic cone can be made out, the posterior end being bluntly rounded. The greatest width is attained at the level of the anterior testis. The cuticle is thick and smooth without either spines or scales.

At the end of the cephalic cone in the fully developed forms is a small circular mouth, surrounded by an oral sucker also circular and measuring from 270$\mu$ to 700$\mu$ in diameter. At a distance of almost 300$\mu$ behind the oral sucker is a large ventral sucker (700$\mu$ in diameter and from 1·54 to 2·8 mm. in length) produced posteriorly into a sac-like prolongation. The ratio between the oral and the ventral sucker is 1 : 3·2 to 1 : 3·4. Immediately following upon the oral sucker is a prepharyngeal sphincter behind which is a globular pharynx 240$\mu$ to 670$\mu$ in diameter. There is no oesophagus. The intestinal caeca appear to arise from the pharynx and pass posteriorly almost to the hinder end of the body in a zigzag manner with two characteristic curves, one in front of the anterior testis between it and the ovary and another between the testes. The thickness is uniform throughout.

The genital pore is immediately anterior to the ventral sucker. The cirrus sac is very characteristic. It is not a cylindrical straight pouch as in other species, but is peculiarly convoluted. It extends from between the shell gland and ventral sucker and passes anteriorly, touching the right border of the latter organ. In a well-grown specimen its width is 700$\mu$ and its length 8·5 mm. The posterior end is at the posterior two-thirds or more of the distance between the shell gland and the hinder border of the ventral sucker, the exact distance depending on its degree of convolution which is more pronounced in adults. In immature forms where the sac is nearly a cylindrical tube, its posterior end almost approaches the shell gland. The testes are the most prominent organs in the body, occupying nearly half its total area, lying one behind the other, and extending posteriorly from the shell gland. They are separated from the posterior border and the sides by the vitellaria. They are very much branched, each with four main branches, two diverging anteriorly and two posteriorly from a central point. In a fully

grown and well extended specimen the anterior testis measures 9 mm. antero-posteriorly and 12·5 mm. laterally, the posterior, 8·5 mm. antero-posteriorly and 12 mm. laterally. In no case, mature or immature, was the anterior testis found to be smaller than the posterior as Rodenwaldt (1909) has stated. The vas efferens from the posterior testis arises from the centre and passes anteriorly under the anterior testis, after which it turns towards the right to pass on the same side of the shell gland. In front of this it curves towards the centre, to meet a little behind the cirrus sac with the corresponding vas efferens from the anterior testis coming from the left side of the shell gland. The vas deferens so formed continues anteriorly for a short distance, then enters the cirrus sac, inside which it immediately swells into a seminal vesicle. This is continued anteriorly in a peculiarly convoluted course to the middle of the ventral sucker, where it is surrounded by the pars prostatica, then as the cirrus, finally opens by the male pore into the genital sinus.

The ovary is small and very much branched, measuring 230$\mu$ by 190$\mu$ in immature specimens and 2·85 mm. by 1·6 mm. in the fully mature forms. It lies to the right side of the body in front of the anterior testis, between it and the uterine coils. This position is liable to very interesting variations. In immature forms the ovary lies almost exactly in the middle of the body, but as the posterior half increases in size faster than the anterior, its centre moves more and more anteriorly as growth proceeds, until at last, in the fully developed specimens, it comes to lie in the anterior two-fifths of the body. From its inner side a short oviduct passes into the shell gland which, in the immature specimens, is somewhat oval measuring 240$\mu$ by 290$\mu$, and in the mature forms almond-shaped, measuring 2·5 mm. by 1·55 mm. It lies in the centre in front of the anterior testis, always in the same position with regard to the ovary. On the ventral side is a spherical receptaculum seminis, measuring in fully developed specimens 650$\mu$ in diameter. In the immature forms no such organ is developed. Laurer's canal is short and opens on the dorsal side. The uterus becomes visible at the shell gland. From thence it passes to the left and again to the right, thus describing several loops between the shell gland and the ventral sucker. As the vagina, it is continued anteriorly by the side of the cirrus sac and dorsally to the ventral sucker to open into the genital sinus

through the female pore to the left of the male aperture. In some of the sectioned specimens, the greater part of the uterus was observed to be filled with spermatozoa. The vitelline glands are well developed and occupy a large area. They extend as lateral bands from the ventral sucker to the posterior end of the body, where both unite in the middle line behind the posterior testis. They consist of numerous round follicles each composed of several small acini. The stout transverse vitelline ducts (140$\mu$ in thickness) pass in front of the anterior testis and join in the middle line to form a common duct which opens into a yolk reservoir (1·78 mm. by 1·17 mm.) dorsal to the shell gland. The eggs are oval, thin-shelled and measure from 140$\mu$ to 162$\mu$ in length, and from 85$\mu$ to 95$\mu$ in breadth.

The description of the adult forms collected agrees with that given by Rodenwaldt (1909) in all respects, except that the anterior testis is larger than the posterior, whereas in specimens described by Rodenwaldt the contrary was the case. This discrepancy is probably due to the amount of contraction undergone by Rodenwaldt's specimens in the process of fixation. Further, his description was based upon only two specimens. More extensive material would possibly have caused a revision of his results with regard to this point. There are, in addition, slight differences of dimensions, but this point cannot be regarded as important, dependent as it is on the age and state of contraction of the worms.

### *TESTIFRONDOSA CRISTATA*, n.g., n.sp. (Plate VII)

About three dozen specimens were found on one occasion in the intestine of *Sus cristatus*. The body of the worm is flat, with almost parallel sides which taper anteriorly, and it is covered with a thick cuticle. At the posterior end is a notch, above which is the excretory pore. The anterior part of the body from the level of the ventral sucker is thickly covered with backwardly directed scales which become more sparse posteriorly until they disappear completely at the level of the anterior testis. The length of the mature worms varies from 6 to 8 mm., the last being specimens extended under a little pressure. In breadth they vary from 2·5 to 3·5 mm., the greatest breadth being attained at the level of the posterior testis.

The circular mouth at the anterior end is surrounded by an oral sucker, 220$\mu$ in diameter. The ventral sucker is much larger than the oral and is drawn out posteriorly into a sac-like prolongation. It is 1·02 mm. long by 0·82 mm. in diameter, the excess of the length over the breadth being due to the sac-like prolongation posteriorly. The ratio between the oral and the ventral sucker is nearly 1 : 4. The usual position of the ventral sucker is with its centre approximately at the anterior fifth of the body.

The prepharynx is small, just behind the oral sucker. No pharyngeal glands were observed. The pharynx is globular with thick muscular walls and a diameter of 180$\mu$. It is followed by an oesophagus 230$\mu$ in length. The intestinal caeca are unbranched and reach almost to the posterior end of the body. Each caecum is not uniform throughout, but narrower near the fork and slightly broader posteriorly.

The excretory vesicle is small and pear-shaped and opens behind by a pore on the dorsal side, a little in front of the notch at the posterior end. It extends anteriorly only for a short distance, and into it open several excretory ducts.

The testes lie centrally in the posterior half of the body one behind the other and are enclosed laterally and posteriorly by the vitellaria. They are the most conspicuous of all the organs in the body and occupy little more than one-fourth of its total length. Their most striking peculiarity is their branched appearance, the anterior one being in the form of a horizontal and the posterior approximately in the form of a vertical cross with the anterior limb slightly lateral. All limbs of the cross are branched irregularly. This branching constitutes the distinguishing characteristic of the genus. The dimensions of the anterior testis are 1·43 by 1·13 mm., and those of the posterior 1·31 by 1·18 mm. The vasa efferentia arise from the centre of the anterior end of each testis, and, after a zigzag course, unite to form a vas deferens opening at the base of the seminal vesicle. This latter is an elongated sac lying at the end of the cirrus sac and occupying the last quarter of its length ; it is continued as a convoluted tube filling three-fourths of the length of the cirrus sac and finally opens into a coiled ductus ejaculatorius ending in a short muscular cirrus. No ductus hermaphroditicus was noticed. Surrounding the cirrus and the ductus ejaculatorius, and

opening into them, are the prostatic glands. The cirrus sac is very much elongated and extends from the intestinal fork to some distance behind the ventral sucker. Its anterior fourth, which lies between the intestinal fork and the ventral sucker, is swollen, as is also the posterior fourth behind the ventral sucker. The middle portion is thinner. The position of the cirrus sac varies according to the state of contraction of the body. Normally it lies dorsal to the ventral sucker, but not infrequently it occurs sometimes to the right and sometimes to the left. The genital pore is central immediately behind the intestinal fork.

The ovary is approximately oval. It is situated on the ventral surface a little to the left of the middle line, between the posterior end of the cirrus sac and the anterior testis. The oviduct is small and runs centrally to the large shell gland, situated between the anterior testis and the ovary. Laurer's canal lies horizontally between the shell-gland and the anterior testis. The receptaculum seminis is ventral, almost in the centre of the body at the level of the ovary ; in entire mounts it is obscured by the shell gland. The vitellaria are lateral and numerous, extending from about half the length of the ventral sucker to the end of the body and occupying nearly two-thirds of the entire length. They consist of numerous small round follicles, each composed of a few acini. They are very narrow anteriorly, but become broader and broader posteriorly until, at a little distance behind the posterior testis, they meet in the middle line. Anteriorly they are marginal to the intestinal caeca, but posteriorly they overlap them. Nearly one-fifth of the posterior portion of the body is filled with these glands. Vitelline ducts arise at the level of the shell gland and open in the centre into a yolk reservoir communicating with the oviduct. Beyond this junction the oviduct is continued as the uterus which, describing six or seven turns between the shell gland and the ventral sucker, continues dorsally as a vagina lined with a thick cuticle. It finally opens into the genital pore by the side of the male genital aperture. The eggs are large, oval and operculated, from 110$\mu$ to 130$\mu$ long by 70$\mu$ to 80$\mu$ broad.

In the branched nature of the testes, the extent and form of the vitellaria, and the possession of a pouch to the ventral sucker, the present form resembles the genus *Fasciolopsis*, but differs from it

in the possession of a receptaculum seminis, and in the extent and position of the cirrus sac. It agrees in all important particulars with the description of the sub-family *Psilostominae*, Lühe, 1909, but differs in the branched nature of the testes: the genus *Psilochasmus*, Lühe, 1909, however, has somewhat deeply lobed testes. As the present form differs in important characters from any existing genus, it is necessary to create a new one for its reception. For this I propose the name *Testifrondosa*, and for the species *T. cristata*.

*Testifrondosa* (n.g.)

DIAGNOSIS :—

Psilostominae : Body covered with scales. Oral sucker smaller than the ventral, latter drawn out posteriorly into a sac-like prolongation. Prepharynx small, pharynx small, globular. Oesophagus short. Intestinal caeca nearly reaching posterior end of the body. Genital pore near intestinal fork. Cirrus sac much elongated, extending beyond ventral sucker and containing vesicula seminalis. Pars prostatica and Laurer's canal present. Testes branched, in posterior half of the body, one behind the other. Shell gland central. Ovary anterior to testes. Receptaculum seminis present. Vitellaria lateral, meeting in the middle line posterior to testes. Uterine coils between shell of gland and cirrus sac. Excretory canal pear-shaped. Eggs large, operculated.

HOST : *Sus cristatus* (Wagner, 1909).

TYPE SPECIES : *Testifrondosa cristata*, n.sp.

### *EURYTREMA DAJII*, n. sp. (Plate VIII)

From thirty to forty specimens of this species were collected in the bile-ducts of a *Bos indicus* also infested with *Paramphistomum cervi* and *Fasciola gigantica*. They were 5 to 6·7 mm. long by 3·5 to 4 mm. broad, and 6·32 mm. thick, coloured red and very sluggish. All were mature. They appeared to be roundish in their natural habitat, but when put in luke-warm water became leaf-like and flattened dorso-ventrally. The body was transparent, so that upon applying a little pressure in the living condition, the whole anatomy could be made out under a low magnification. The body is covered with a thin cuticle, bearing small square scales 20$\mu$ to 50$\mu$, very thinly distributed and absent from the edges. The maximum

breadth is reached at the level of the ovary or a little behind it, thence the body narrows towards the anterior extremity, ending, however, rather bluntly owing to the presence of the oral sucker. Above this the margin of the body projects in the shape of a thick lip. At the posterior end is a conspicuous tongue-like appendage—the caudal appendage. A little behind the anterior extremity is a circular mouth surrounded by an oral sucker which, in most cases, is round, but sometimes has its antero-posterior diameter slightly the bigger. On an average the oral sucker is 750$\mu$ in diameter. The ventral sucker lies in the anterior half of the body: its size in some cases is equal to, and in others slightly larger than that of the oral sucker, the greater diameter being on the average 850$\mu$. The ratio between the two suckers is 1 : 1·3; the distance between their centres is a little more than one-third the total length of the whole body. The pharynx is small, 250$\mu$ by 220$\mu$ and lies immediately behind the oral sucker. Following it is a very short oesophagus approximately 50$\mu$ long. This immediately bifurcates into two intestinal caeca which diverge and pass along the sides of the body in a sinuous course that becomes specially accentuated behind the testis. Their blind ends terminate a little in front of the caudal appendage, about 800$\mu$ from the posterior end of the worm. The excretory pore is at the extreme posterior end, on the top of the caudal appendage. It leads in to an excretory vesicle which becomes bigger as it passes anteriorly and divides into two branches at about half-way between the posterior border of the ventral sucker and the hinder end of the body. These branches pass slightly anteriorly to each side and cross over the intestinal caeca. At the level of the ovary each divides again into anterior and posterior branches which can be traced almost to the ends of the body.

The genital pore is a little behind the intestinal fork, nearer the oral than the ventral sucker. The cirrus sac is elongated, much wider anteriorly and tapering behind, and extends from a little behind the intestinal fork to a little in front of the ventral sucker. It is inclined slightly to the left of the middle line. In a fully extended specimen it is 1 mm. long by 0·38 mm. broad, but the size varies greatly according to the degree of body contraction. The testes almost touch the intestinal caeca behind the ventral sucker. They measure 400$\mu$ to 560$\mu$ in length and 220$\mu$ to 350$\mu$ in breadth.

Their position varies greatly ; sometimes they are quite symmetrical, but not infrequently the right is a little in advance of the left or *vice versa*. The margin is lobed, sometimes deeply, sometimes slightly, the number of lobes being usually four. From the anterior lobe or from the lobe directed towards the ventral sucker of each testis, arises a vas efferens which runs inwardly towards its fellow on the other side and joins with it to form a vas deferens at the base of the cirrus sac. The vas deferens then passes into the cirrus sac where, after a short interval, it widens into a vesicula seminalis. The vesicula seminalis continues as a convoluted tube filling up nearly two-fifths of the posterior part of the cirrus sac, then, as a ductus ejaculatorius and finally as a muscular cirrus, opens into the genital sinus by the male aperture. The ductus ejaculatorius is surrounded by the pars prostatica.

The ovary is small, 220$\mu$ to 300$\mu$ by 170$\mu$ to 250$\mu$. It lies in the posterior half of the body, a little to the left of the middle line and a short distance behind the testes. In a well extended form it appears to be trilobed, in others its shape varies, sometimes being oval, and sometimes elongated transversely. From its inner side a short duct passes into the shell gland, 200$\mu$ by 130$\mu$, near the ovary. On the dorsal side and overlapping the ovary is a small round receptaculum seminis, 190$\mu$ by 160$\mu$. Laurer's canal proceeds from the ovary anteriorly towards the ventral sucker, a little behind which it terminates. In a fully extended condition it is 300$\mu$ long by 30$\mu$ wide. Its most striking characteristic is the absence of an external pore. In living specimens its blind nature can be ascertained by the movements that it makes when subjected to slight change of pressure : even in sections no opening to the exterior could be discovered. In this respect the present species differs from all others of the same genus.

The uterus becomes visible after the shell gland. It first coils posteriorly, then passes anteriorly between the right testis and the ventral sucker to continue, still coiled, on the right side of the latter, where it joins a long, thin, muscular vagina. This passes lateral to the cirrus sac and opens by the female aperture on the anterior side of the genital atrium. The uterine coils are numerous and nearly overlap the intestinal caeca : anteriorly they are limited by the testes and the ventral sucker : posteriorly they may end at the

termination of the intestinal caeca, but more often project beyond them and occupy a portion of the caudal appendage. The uterine coils are not in well-defined loops but appear to be scattered branches. Sometimes, in a particular state of contraction, the descending and ascending parts of the uterus pass so near one another in the middle line, that the appearance is that of a central stem with many lateral branches.

The vitelline glands are not well developed. They consist of from ten to thirteen groups on each side, each composed of numerous slender acini. In fully extended specimens the groups lie one behind the other outside the intestinal caeca : they are sometimes round, but are liable to much variation in contracted specimens ; in these the linear arrangement of the groups is disturbed, so that they lie irregularly outside the intestinal caeca, occasionally with the follicles pushed below each caecum. The individual groups of follicles become sometimes so much stretched that the acini are arranged in irregular lines, usually commencing on the outer border of the testis and ending at about half-way between the posterior border of the ventral sucker and the base of the caudal appendage. Sometimes the glands on the same side as the ovary extend further anteriorly than those of the opposite side, and sometimes *vice versa*. Two smaller vitelline ducts, anterior and posterior, from each gland unite to form a transverse vitelline duct which, arising from the centre, passes internally to meet with its fellow from the other side and open into the shell gland. No yolk reservoir was noticed. The eggs are small, operculated, 32$\mu$ to 40$\mu$ long by 22$\mu$ to 27$\mu$ broad.

Up to the present, seven species of the genus *Eurytrema* have been described, one of them, *Eurytrema crucifer* (Nicoll, 1914), later being placed by Kossack (1910) in his new genus *Paradistomum*. The description of *Eurytrema parvum* (Senoo, 1907) is not accessible to me in India. From the table given below it may be seen that the present species is allied to *Eurytrema pancreaticum* (Janson, 1889) with which it agreees :

(i) in the possession of a caudal appendage ;
(ii) the position of the genital aperture ;
(iii) the extent and the number of groups of vitellaria, and
(iv) the nature and disposition of the uterus,

but differs

(i) in the possession of cuticular scales ;
(ii) the ventral sucker being slightly larger than the oral ;
(iii) the position of the cirrus sac, and
(iv) the blind nature of Laurer's canal.

These points seem to me of sufficient importance to justify the creation of a new species for which I propose the name *Eurytrema dajii.*

SPECIFIC DIAGNOSIS.

*Eurytrema :* 5 to 6·7 mm. by 3·5 to 4 mm. Cuticle covered with scales. Caudal appendage well developed. Ventral sucker slightly larger than oral. Genital pore posterior to intestinal bifurcation. Cirrus sac not reaching anterior margin of ventral sucker. Vitellaria elongated, 10 to 13 groups, extending from testis to half the distance between ventral sucker and proximal end of caudal appendage. Laurer's canal with no external opening. Uterus passing anteriorly, lateral to ventral sucker : uterine coils asymmetrical, not crossing intestinal caeca.

HOST : *Bos indicus* (bile ducts).

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A comparative Table of the species of the genus *Eurytrema*

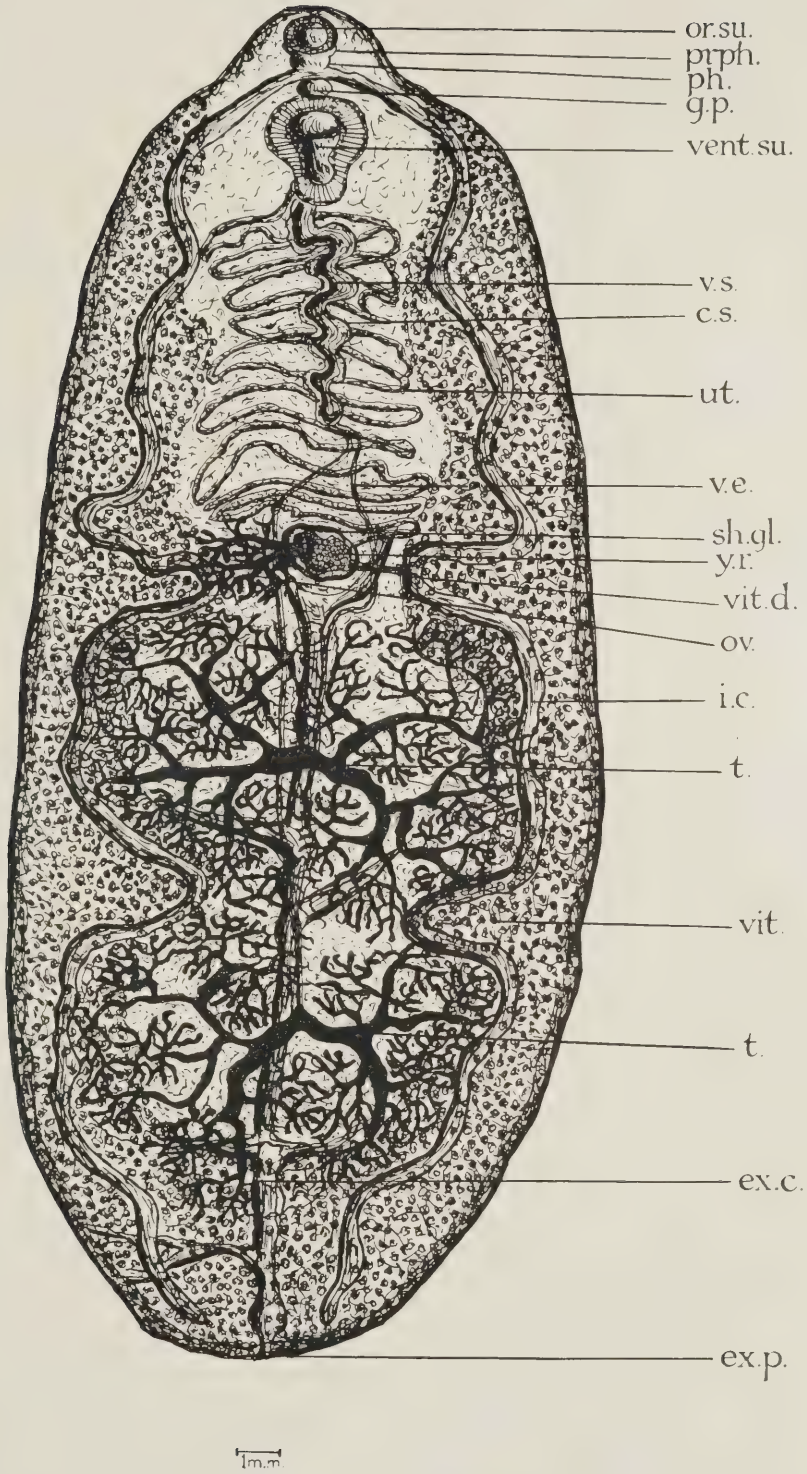
| Species ... ... ... | *E. dajii*, n.sp. | *E. pancreaticum* (Janson, 1889) | *E. coelomaticum* (Giard and Billet, 1892) | *E. concinnum* (Braun, 1901) | *E. brumpti* (Railliet, Henry, and Joyeux, 1912) | *E. satoi* (Kobayashi, 1915) |
|---|---|---|---|---|---|---|
| Host ... ... ... | *Bos indicus*, bile ducts | Bovidae, pancreatic ducts | Bovidae, pancreatic ducts | *Vivera zibetha*, gall bladder | Chimpanzee, bile and pancreatic ducts | *Macacus cynomolgus*, pancreatic ducts |
| Size ... ... ... | 5-6·7 by 3·5-4 mm. | 13-14 by 6·5-7 mm. | 7-10 by 4-5·5 mm. | 2·7-3 by 1·6 mm. | 3·5-4 by 1·8-2·3 mm. | 6-6·5 by 2-3 mm. |
| Scales ... ... ... | Present | Absent | Absent | Absent | Very fine | Absent |
| Caudal appendages ... | Present | Present | Present | Absent | Absent | Less conspicuous |
| Size of suckers ... ... | Ventral slightly larger | Oral larger | Both equal | Ventral larger | Ventral larger | Ventral slightly larger |
| Genital pore ... ... | Behind bifurcation of intestine | Behind bifurcation of intestine | Behind bifurcation of intestine | Before bifurcation of intestine | Before bifurcation of intestine | Behind bifurcation of intestine |
| Cirrus sac ... ... | Not reaching anterior border of ventral sucker | Reaching anterior border of ventral sucker | Reaching anterior border of ventral sucker | Not reaching anterior border of ventral sucker | Not reaching anterior border of ventral sucker | Not reaching anterior border of ventral sucker |
| Vitellaria ... ... | Elongated, 10 to 13 groups, from testes to one half the distance between ventral sucker and caudal appendage | Elongated, 10 to 12 groups, from testes to one half the distance between ventral sucker and caudal appendage | Elongated, 6 to 8 groups posterior to testes | Round, 6 groups | Round, 11 to 14 groups behind ovary | Elongated, 3 to 4 groups behind ovary |
| Laurer's Canal ... ... | With no external pore | With external pore | With external pore | With external pore | With external pore | With external pore |
| Position of uterus ... | By the side of ventral sucker | By the side of ventral sucker | By the side of ventral sucker | Above ventral sucker | Above ventral sucker | By the side of ventral sucker |
| Uterine groups ... ... | Anterior uterine groups asymmetrical; do not cross over intestinal caeca | Anterior uterine groups asymmetrical; do not cross intestinal caeca | Anterior uterine groups asymmetrical; do not cross intestinal caeca. | Anterior uterine groups slightly asymmetrical; do not cross intestinal caeca | Anterior uterine groups asymmetrical; cross intestinal caeca | Cross intestinal caeca |

*Eurytrema parvium* (Senoo, 1907) is not included in the above table.

## EXPLANATION OF PLATE VI

Ventral view of *Fasciolopsis füllebornii*

| | | | |
|---|---|---|---|
| *c.s.* | Cirrus sac. | *sh.gl.* | Shell gland. |
| *ex.c.* | Excretory canal. | *t.* | Testis. |
| *ex.p.* | Excretory pore | *ut.* | Uterus. |
| *g.p.* | Genital pore. | *v.s.* | Vesicula seminalis. |
| *i.c.* | Intestinal caecum. | *v.e.* | Vas efferens |
| *or.su.* | Oral sucker. | *vent.su.* | Ventral sucker. |
| *Ov.* | Ovary. | *vit.* | Vitellaria. |
| *ph.* | Pharynx. | *vit.d.* | Vitelline duct. |
| *prph.* | Prepharynx. | *y.r.* | Yolk reservoir. |

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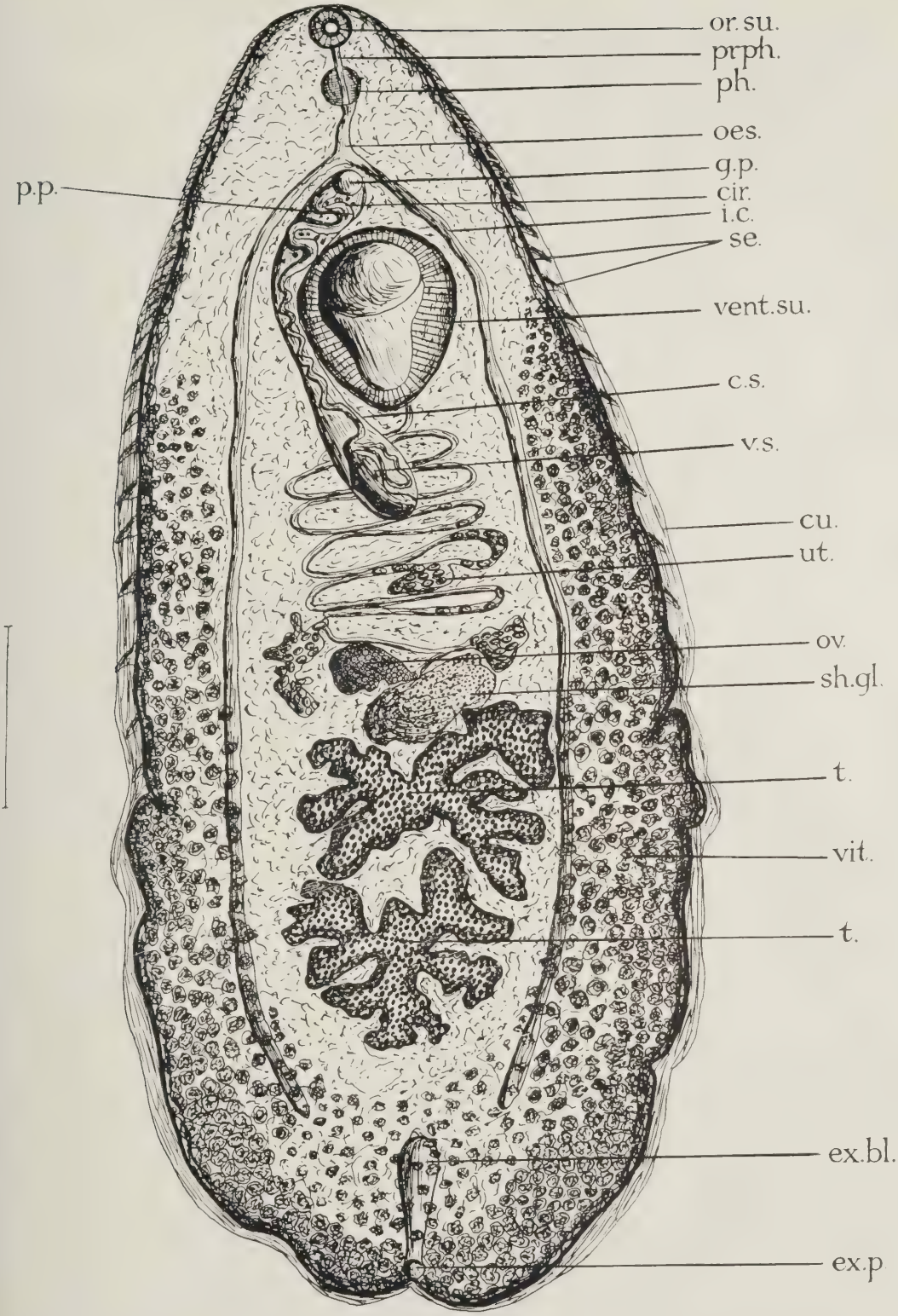
## EXPLANATION OF PLATE VII

Dorsal view of *Testifrondosa cristata.*

| | | | |
|---|---|---|---|
| *c.s.* | Cirrus sac. | *p.p.* | Pars prostatica. |
| *cir.* | Cirrus. | *prph.* | Prepharynx. |
| *cu.* | Cuticle. | *ph.* | Pharynx. |
| *ex.bl.* | Excretory bladder. | *sc.* | Scales. |
| *ex.p.* | Excretory pore. | *sh.gl.* | Shell gland. |
| *g.p.* | Genital pore. | *t.* | Testis. |
| *i.c.* | Intestinal caecum. | *ut.* | Uterus. |
| *Oes.* | Oesophagus. | *v.s.* | Vesicula seminalis. |
| *or.su.* | Oral sucker. | *vent. su.* | Ventral sucker. |
| *ov.* | Ovary. | *vit.* | Vitellaria. |

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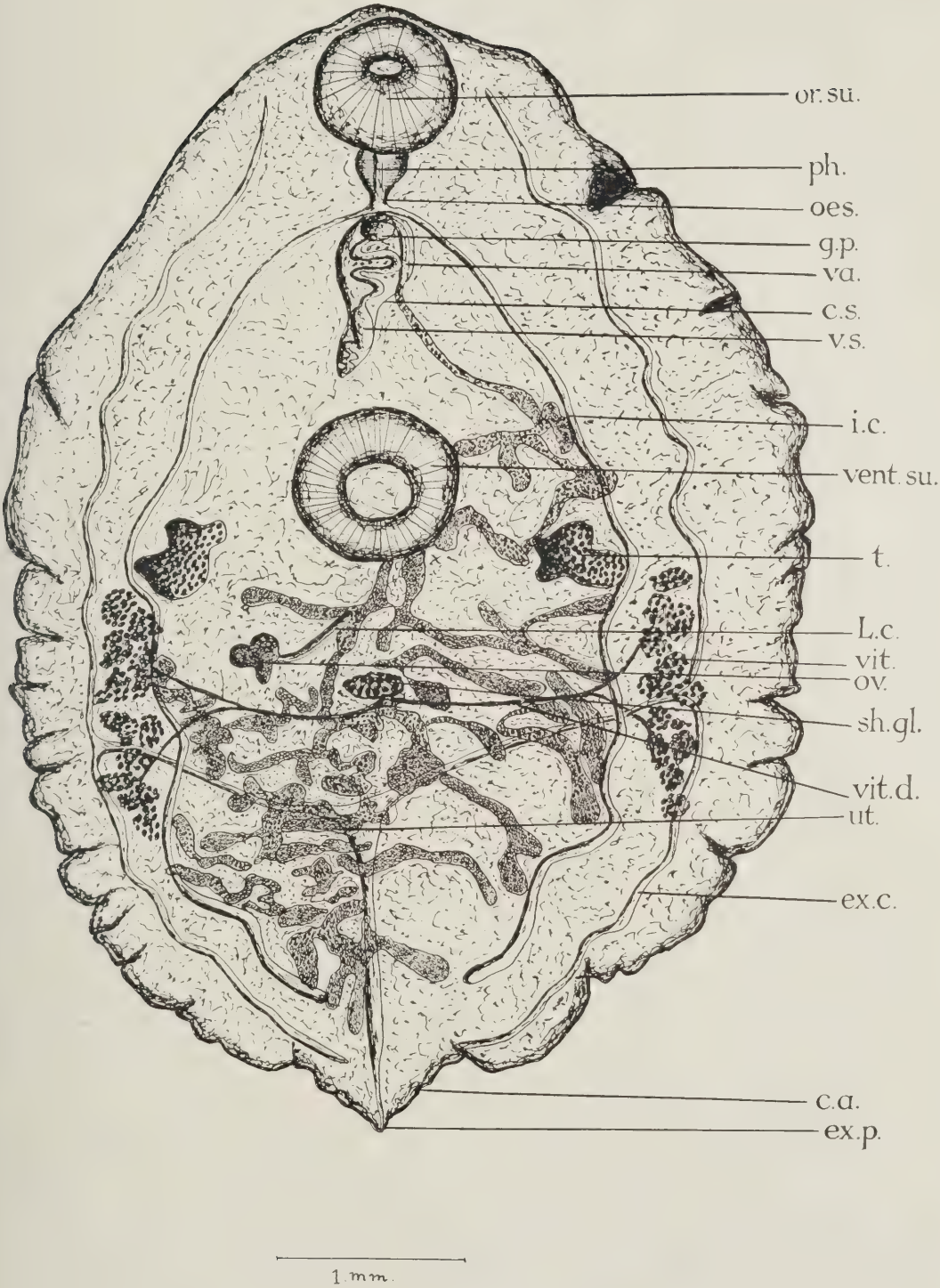
## EXPLANATION OF PLATE VIII

Dorsal view of *Eurytrema dajii*.

| | | | |
|---|---|---|---|
| *c.s.* | Cirrus sac. | *ov.* | Ovary. |
| *c.a.* | Caudal appendage. | *ph.* | Pharynx. |
| *ex.c.* | Excretory canal. | *sh.gl.* | Shell gland. |
| *ex.p.* | Excretory pore. | *ut.* | Uterus. |
| *g.p.* | Genital pore. | *v.s.* | Vesicula seminalis. |
| *i.c.* | Intestinal caecum. | *va.* | Vagina. |
| *L.c.* | Laurer's canal. | *vent.su.* | Ventral sucker. |
| *oes.* | Oesophagus. | *vit.* | Vitellaria. |
| *or.su.* | Oral sucker. | *vit.d.* | Vitelline duct. |

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# OBSERVATIONS ON THE CAUSAL ORGANISM OF RAT-BITE FEVER IN MAN

BY

ANDREW ROBERTSON, M.B., Ch.B.

(GROCERS' RESEARCH SCHOLAR AND ASSISTANT IN THE DEPARTMENT OF PROTOZOOLOGY, LONDON SCHOOL OF TROPICAL MEDICINE.)

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In June, 1923, a patient was admitted to the Hospital for Tropical Diseases giving a typical history of rat-bite fever, and showing all the clinical signs of the disease. The clinical aspects of the case have already been published in detail by Dr. G. C. Low (1924), Senior Physician, Hospital for Tropical Diseases, and the following observations have been made on a strain of the parasite derived—thanks to the courtesy of Dr. Low—from this patient.

On 11th June, 1923, two rats and two mice were inoculated with citrated blood from the patient, who was then at the height of a pyrexial attack, the former being given about 3 c.c. each, and the latter 1·5 c.c. all intraperitoneally. Of the four animals inoculated, only one mouse showed the parasite in its peripheral blood. These animals had been bred in the laboratory, and examinations of their blood for several days previous to the inoculation failed to reveal parasites. The appearances and behaviour of the parasite in this mouse were such as to indicate that the organism differed in many respects from the members of the genera *Spirochaeta*, *Leptospira*, etc. to which it has at times been referred, and accordingly search was made through the literature for previous descriptions.

## HISTORICAL

Vandyke Carter (1887) published a description of a *Spirillum*, which he discovered in the blood of a rat (*Mus decumanus*), in India. His attention was first drawn to the possible presence of some parasite by a quick, twirling movement of the red blood corpuscles in

fresh blood, and, on further investigation, he found that this was due to an organism, which he described as an

'extended and uniformly slender filament of clearly spiral construction, having a length commonly somewhat less than the diameter of a blood disc, but varying from $5\mu$ to $9\mu$ . . . . and according to its length presenting from four to eight close spiral turns.'

He was unable to make out the presence of flagella at the ends. The movement of the organism was very active, and consisted of

'rotation round the long axis, propulsion either forward or backward, and occasionally an energetic twisting or lashing.'

From its morphology, movements, and behaviour to reagents Carter concluded that the organism in question was

'a bacterium belonging to the genus spirochaete . . . . such as from its small dimensions might be named provisionally *Spirillum minor*.'

Attempts to inoculate the organism into another rat and also into a monkey were not successful.

There seems to be little doubt that the organism, which Carter found in the blood of the rat, is the same as that which causes rat-bite fever in man.

Thereafter there occur numerous mentions of similar organisms, which were found in the blood of animals. Lingard (1899) found a small *Spirillum* in the blood of a bandicoot (*Mus giganteus*). This he was able to inoculate into rabbits and guinea-pigs, but in the latter it was possible to demonstrate the organism only in the final stages of the infection. While Lingard's description of the parasite is lacking in detail, its behaviour in inoculated animals is similar to that of the virus of rat-bite fever as reported by various workers.

Borrel (1905) found a spirochaete in cancer growths of mice. This work was confirmed by Calkins and Clowes (1905), Deetjen (1906) Tyzzer (1906-07), and Gaylord (1907). There is a marked similarity between the organisms described by these workers, although the measurements varied considerably.

Wenyon (1906) described a spirochaete in the blood of mice which he named *Spirochaeta muris*. This he considered to be identical with the spirochaete found by Borrel, but with reference to the organism of Carter he states that

'since the morphological characters of spirochaetes are not sufficient to establish the identity of any form it is necessary to rely on other characters, notably their behaviour

in various hosts, and their pathogenic or other action. As nothing is known of Carter's *Spirillum* of the rat apart from its morphology, the spirochaete of the mouse must be considered to be new to science.'

Morphologically, Wenyon's *Spirochaeta muris* closely resembled Carter's *Spirillum*, and the characteristic movements were also present. Wenyon was unable to demonstrate flagella, but suspected that they might be present, since all the movements of the organism seemed to indicate their presence. The spirochaete was easily inoculable into mice, but could not be demonstrated in guinea-pigs.

Breinl and Kinghorn (1906) isolated a spirochaete from the same source as Wenyon, viz.:—a mouse inoculated with *Trypanosoma dimorphon*, which was sent to them from Paris. They also found a similar spirochaete in the blood of a wild mouse. The parasite was smaller in size than that described by Carter, and accordingly, since they considered it to be a distinct organism, they named it *Spirochaeta laverani*.

MacNeal (1907) demonstrated the presence of flagella at the ends of the spiral in a strain of the parasite isolated from one out of thirty-nine rats (*Mus decumanus*), caught at Morgantown. He considered this organism to be identical with those of Carter, Wenyon, Breinl and Kinghorn, and Nicolle and Compte (in a bat). Despite the fact that he thought his organism to be the same as that of Carter, and that terminal flagella had been seen, MacNeal adopted Wenyon's name *Spirochaeta muris* (var. *Virginiana*).

In man, rat-bite fever has long been recognised as a definite clinical entity. Early accounts of the disease were given by Wilcox (1840) in America, Millot-Carpentier (1884) in France, Peña y Maya (1885) in Spain, Miyake (1889) in Japan, and Horder (1909) in England. While it was soon recognised that the disease depended on the introduction of some virus into the wound caused by the bite of the rat, nevertheless it was comparatively recently that it was associated with a definite organism. That more than one disease may be inoculated into man by the bite of a rat is practically certain, and this led to some confusion, in that some of these diseases were mistaken for the fever of the relapsing type, which is the clinical entity known as rat-bite fever. Shikami (1907) attributed rat-bite fever to a member of the Telosporidia; Middleton (1910) thought that the causative organism was a *Diplococcus*;

Ogata (1911), who produced rat-bite fever experimentally by allowing a rat to bite guinea-pigs, considered that the disease was due to an *Aspergillus*; Proescher (1911) described *Bacilli*, which were very numerous in the base of the wound; Schottmüller (1914), Blake (1916) and Tileston (1916) isolated from the blood of patients, who had been bitten by rats and by a South African squirrel, a *Streptothrix*, which was named *Streptothrix muris ratti*. With reference to this last, it is of interest that Tunnicliff (1916) isolated a similar *Streptothrix* from rats with broncho-pneumonia. Hata (1912), Surveyor (1913) and Dalal (1914) reported cures by the use of salvarsan, arguing that, on account of the periodicity of the fever and the peculiar eruption, the infecting organism might be allied to the *Spirochaetae*.

It was, however, left to Futaki (1915) and his co-workers to discover the causative organism of rat-bite fever in man. This was a spiral organism having flagella at the ends, varying in length from $2\mu$ to $5\mu$, and when the flagella are included from $6\mu$ to $10\mu$. They named it *Spirochaeta morsus muris*, and considered it to be of the nature of a *Treponema*. The movements of *S. morsus muris* were very rapid, 'resembling those of a vibrio.' It stained readily with Giemsa's stain. Animal experiments were successful; monkeys, mice, house rats and white rats were inoculated with positive results. These authors also claim to have cultivated the parasite on Shimamine's medium, but the culture organisms, as described and figured, differed very considerably from the forms found in man and animals. In cultures, forms appeared up to $19\mu$ in length, and having at times as many as nineteen coils. The coils or spirals were wider than those of the blood forms—one coil to $2\mu$, whereas the blood forms had one coil to $1\mu$. Whether it was possible successfully to inoculate these cultural forms into experimental animals is not stated. Further mention of these cultural forms will be made later.

Futaki and his associates were of the opinion that the organism, which they had isolated, differed in certain respects from those previously described as occurring in the blood of mice, rats and other animals, and further, that, as no connection between these organisms and rat-bite fever in man had been demonstrated, they were justified in considering that the parasite was a new species.

Ishiwara, Ohtawara and Tamura (1915) dealt with certain

experimental aspects of the question. They allowed infected rats to bite guinea-pigs, thus confirming the work of Ogata, and studied the course of the disease in these animals. They discussed the similarity of Wenyon's *Spirochaeta muris* and the *Spirochaeta laverani* of Breinl and Kinghorn to *S. morsus muris*. Ishiwara and his associates did not succeed in cultivating it.

Kaneko and Okuda (1917), dealing with the organism in man, came to the conclusion that the long and short forms described by Futaki were not diverse in type. The short forms were found in the peripheral blood, but the long forms—similar to the cultural forms of Futaki—they found in renal casts, in the tissues of the kidneys and in the suprarenals. This change in the morphology they attribute to the formation of immune bodies in the patient, and are of the opinion that the long forms are really old and degenerate individuals.

Ido, Ito, Wani and Okuda (1917) obtained blood serum from three human patients recovering from rat-bite fever. The effect of this serum they tested on spirochaetes in the blood of guinea-pigs, which had been infected from a wild rat, *Mus decumanus*, and found that the organisms were killed, whereas in controls treated with serum from patients who had not had rat-bite fever, and also with isotonic salt solution, the spirochaetes remained actively motile and relatively numerous. This is of interest in that, in addition to illustrating the action of immune serum on the parasites, it suggests a certain degree of association between the causative organism of rat-bite fever in man, and similar parasites found in the peripheral blood of mice and rats.

Row (1917) isolated a spiral organism from a case of human rat-bite fever in India. This was rather smaller in size than that described by the Japanese workers, $3\mu$ to $5\mu$ in length, and Row thought it a distinct species. Later, in 1922, Row named his organism *Spirochaeta petit*. Row found that

> 'the broad distinction made by Futaki into the long and short forms according to the situation from which the virus is derived—the long forms from lymphatic nodules and short ones from the peripheral blood—does not hold good in Bombay.'

The Bombay virus was not fatally virulent to guinea-pigs and rats. Row came to the conclusion that the number of spirochaetes in the organs of infected animals was not greater than could be accounted

for by the blood supply to these organs, and that such as were found were uniform in type with those present in the peripheral blood. The Bombay virus was shorter than that described by the Japanese workers, and was more slender than English strains. The fact, too, that at first the flagella of the Bombay strain were not seen, was an additional reason why Row should place his spirochaete in a new species. Parmanand (1923), however, demonstrated the terminal flagella in this strain, and came to the conclusion that it was similar to the *Spirochaeta morsus muris* of Futaki.

Izumi and Kato (1917), by serological methods, proved the relationship of the spirochaete found in cases of cat-bite disease to that of rat-bite fever.

Manson-Bahr (1922), in 'Manson's Tropical Diseases,' referred the causative organism of rat-bite fever to the genus *Leptospira*, and retained the specific name *morsus muris*. His reasons for making this alteration in the nomenclature were not stated. Sangiorgi (1922) suggested the generic name *Treponemella* for the rat-bite virus.

Numerous reports of cases of rat-bite fever have appeared in the literature since the discovery of the parasite by Futaki and his associates, and the foregoing is not intended to be an exhaustive survey of the literature on the subject. As many of those communications, which have a direct bearing on the parasite, its morphology, nomenclature, etc., as possible, have been mentioned, but many other papers on the subject will be found in the list of references.

## THE PRESENT CASE

While the patient was having one of his periodical bouts of fever, specimens of his blood, saliva, and urine were examined. In the blood, both in stained preparations and in fresh preparations examined with dark ground illumination, no organisms were found. In the urine, also, the results of the search were negative. The saliva proved to have the usual number of spirochaetes of all kinds, but there was also present an undoubted *Spirillum*, which, however, was so scarce that accurate observations could not be made. At the time of the examination the patient showed the typical, dark purplish-red, exanthematous rash, especially over the lower ribs

and upper part of the abdomen on the right side. One of the most conspicuous patches of the rash was chosen, and its surface scarified. Gentle pressure was applied to the scarified area, and preparations of the exuded serum were examined with the dark ground. In one specimen only were the organisms found. Stained films from this source were negative.

A strain of the parasite was isolated in the blood of mice, which had been inoculated with citrated blood from the patient.

## MORPHOLOGY

The body of the organism, which is coiled into a more or less perfect spiral, is rigid. Occasionally, under certain circumstances, the body may be bent, but as soon as the factors which cause the bending are removed, it returns to the original state. In comparison with the members of the genus *Spironema* this rigidity is most striking. The *Spironemata*, in addition to the general flexibility of their bodies, progress by means of an undulating movement, which passes from one end of the body to the other ; the rat-bite organism, on the other hand, retains its fixed shape during movement, although in dark ground preparations the rapid spinning of the body may simulate a wave-like mode of progression. At each end of the spiral body are flagella, usually single but sometimes multiple, and their function seems to be not so much to propel the organism, as to produce the spinning movement of the body, which then passes through the fluid much in the same manner as a screw enters wood. Movement, which is very rapid, is usually in more or less of a straight line, but sometimes consists of sudden dashes to and fro, or in any direction, when either end of the body may be, for the time being, the anterior. Lashing movements of the body are also seen, but there is a possibility that this may be due to the artificial conditions under which the observations are made, such as the fixation of the flagellum at one end to the under surface of the cover glass, to a group of red blood corpuscles, etc.

With regard to the size of the parasite considerable variations have been noted. Thus it varies in different animals, and also in the same animal from day to day. There appears to be a certain tendency to uniformity of size in any given animal on any given day, by which

is meant that one day the preponderating forms will be, for example, long forms, whereas the following day, or subsequently, the majority observed will be shorter or medium-sized. The largest forms seen were between 14$\mu$ and 15$\mu$ including the flagella, *i.e.*, slightly more than double the diameter of the red blood corpuscles of the mouse, and, when body length only is considered, the maximum measurements were between 9$\mu$ and 10$\mu$. It should be noted that the length of the flagella is by no means proportionate to the dimensions of the body, as small forms with a body-length of only 2·5$\mu$ may have flagella as long as individuals with a body-length of 8$\mu$ or thereby. The smallest individuals measured 3·25$\mu$, but these had a flagellum at one end only. The smallest body-length was 1·5$\mu$. The average size was between 3$\mu$ and 5·5$\mu$, and including the flagella between 6$\mu$ and 8$\mu$.

The number of coils or turns of the body varies from one-and-a-half to eight or nine. The width of the body, owing to its spiral construction, is extremely difficult to ascertain with accuracy. Fixed and stained preparations are of little value in this respect, as the degree of distortion is very great. The average width is about 0·2$\mu$, and the width of the spiral as a whole about 0·7$\mu$.

Division takes place by transverse binary fission of the body into two, more or less equal portions. A constriction appears about the middle of the body, and this gradually deepens until the two parts are connected by the merest thread. Certain peculiarities of movement may be seen in these dividing forms owing to the different alignment of the two halves. Finally, the two portions separate, giving rise to two new individuals, which, at first, have flagella at one end only. Later a flagellum appears at the other end. It seems probable that the fully developed organism has a flagellum at each end, and that those forms with flagella at one end only are the products of division. In stained preparations it is quite common to find the flagella at each end to be multiple, but this seems to be more the result of the fixation than a characteristic of the parasite, as, in fresh specimens in which the movement has slowed, it has only rarely been possible to demonstrate more than one flagellum. For some reason, the flagella are very difficult to stain, and even using the same technique, constant results cannot be obtained. Several stains have been tried and various methods of fixation, but the best results

have been obtained with slight modifications of Leishman's and Giemsa's stains.

Futaki and his co-workers described two forms of the parasite, the first as found in the peripheral blood, the second longer forms in cultures. Kaneko and Okuda mentioned similar long forms, which they found in the kidneys, suprarenals and renal tube casts, and these they interpreted as older and somewhat degenerate individuals. Morphologically, there is a great difference between the cultural forms and the blood forms, the former being as many as 19$\mu$ in length and having coils twice as wide as the blood forms, and indeed this variation, especially in view of the fact that no one has succeeded in cultivating the rat-bite virus since Futaki, is so marked that it is necessary to bear in mind the possibility that in the infected animals and cultures, there were spirochaetes other than that of rat-bite fever. In the present case careful search was made in the tissues of animals which were killed during their convalescence from the disease, and also at various stages in the course of the infection, with the result that no true spiral organisms were found other than the forms described above as occurring in the peripheral blood. Hogue (1924), working on *S. eurygyrata*, has shown that the cultural forms of a spirochaete may differ markedly in their morphology from the forms found under natural conditions, but even this is insufficient to explain the difference in length, shape, structure, etc., between the blood forms and Futaki's cultural forms. It is significant, also, that it was not possible to inoculate successfully experimental animals with the cultures.

## CULTIVATION EXPERIMENTS

In the present case all attempts to cultivate the virus have so far proved unsuccessful. N.N.N. (Novy, MacNeal, Nicolle), Noguchi's and Shimamine's media and a dilute blood agar were the chief media used. These were incubated at different temperatures but without result. The cultural experiments are being continued.

## ANIMAL EXPERIMENTS

Of four animals inoculated from the patient, only one mouse showed the parasite in its peripheral blood on the seventh day after inoculation. The other mouse and the two rats never showed

parasites. Mice proved to be most convenient animals in which to carry on the infection, because the *Spirilla* appeared in such relatively large numbers in their blood, and also because they seemed to be little incommoded by the disease. The average length of time which elapsed after inoculation and before parasites appeared was five days, and the infection reached its maximum intensity, so far at least as numbers were concerned, on the ninth day after inoculation. It was quite common, however, for the parasites to show up in fair numbers about the fifth day, disappear the following day, and return in increased numbers on the seventh or eighth day, when they remained constantly present for some weeks. No explanation of this temporary disappearance is known. The shortest period before *Spirilla* could be detected was three days, and the longest fourteen days. Having so quickly reached its maximum intensity the infection gradually declined, the numbers in the blood becoming less and less, until finally they disappeared entirely. As a rule, it is possible to recover the strain by inoculating the blood of the convalescent mouse, even after the *Spirilla* cannot be demonstrated microscopically owing to their scarcity, into a fresh animal. The infection usually persists for the matter of six weeks or so, but, in this instance at least, considerable trouble has been experienced by the mice dying from intercurrent affections.

Guinea-pigs, when inoculated with this strain, showed symptoms of disease. A few days after inoculation, usually about two-and-a-half to three days, there was a definite rise in temperature, which was followed at irregular intervals by similar febrile attacks. During this period, which usually lasted between twenty-one and twenty-four days, the animals were definitely ill, as shown by loss of appetite, poor condition of the fur, general weakness, etc. At the end of this time the disease seemed to reach its most crucial point, and the guinea-pigs were very ill indeed, but this was followed by a gradual convalescence, and in no instance did the animals die from the infection.

It was not possible to demonstrate the *Spirilla* in the blood of the guinea-pigs at any stage, nor were mice successfully inoculated with their blood. These findings are of interest in comparison with those of other workers. Certain strains have proved to be lethal to guinea-pigs, whereas others have caused them little inconvenience ;

some strains have shown parasites in the peripheral blood over a comparatively long period, while others have only appeared immediately before the death of the animal or not at all. One of the guinea-pigs was inoculated subcutaneously in the leg, and this was followed by a painless, oedematous swelling, which persisted until the twenty-first day after. This animal also showed symptoms of the generalised disease.

After the guinea-pigs had recovered, it was found that their serum, when mixed in equal quantities with the blood of mice containing *Spirilla*, rendered the parasites immobile in twenty minutes, whereas in control experiments, the blood of the mice being mixed with serum from uninfected guinea-pigs, with serum from guinea-pigs with various strains of trypanosomes, and with normal saline, the organisms were as actively motile after two hours as they appeared to be when the blood was freshly drawn. The immune bodies in the serum do not seem to be very powerful, since, in dilutions less than about one in four, their effect on the *Spirilla* was very slight. So far no signs of agglutination of the parasites have been seen, although this phenomenon has been described. Mouse blood containing *Spirilla* was mixed with equal parts of immune guinea-pig serum and allowed to stand for fifteen minutes, and was then inoculated into a fresh mouse. Mice, which were inoculated at the same time with untreated blood, showed a heavy infection in five days, whereas this one did not show infection till a considerable time after, and even then the parasites were so few in number that it was only by careful search that they could be demonstrated.

Of the two rats which were inoculated with the blood from the human patient, one died in a few days, while the other never showed parasites in its blood or any signs of disease. It has been possible to find parasites in scanty numbers in the blood of young inoculated rats, but, unless the rats were very young indeed, the *Spirilla* never became evident.

## DISCUSSION

It has been suggested by several workers that the rat-bite fever organism is more closely allied to the genus *Spirillum* than to the *Spirochaeta*, and that is the opinion arrived at by the study of the present strain.

Dobell (1918) discussed the nomenclature of the spirochaete of syphilis. He pointed out that this parasite was morphologically so different from the type species of the genus *Spirochaeta*, viz. :— *S. plicatilis*, that it could not be placed in the same genus. The rat-bite fever organism, with its non-flexible body, terminal flagella and absence of an axial filament, is even more divergent in character, and accordingly cannot be referred to the genus *Spirochaeta*.

For practically the same reasons the rat-bite organism cannot be placed in the genus *Spironema*, the type species of which is *S. pallidum* (Schaudinn, 1905) Vuillemin, 1905.

Manson-Bahr (1918) named the rat-bite fever parasite *Leptospira morsus muris*. He did not state his reasons for thus altering the generic name, but it seems probable that he was influenced, firstly, by the cultural forms of Futaki, which, as figured, have some resemblance to *Leptospira*, and, secondly, by the forms described by Kaneko and Okuda in tissues, renal casts, etc. Now it has been shown by Hogue (1922), working on *S. eurygyrata*, that the cultural forms of a spirochaete may differ from the forms found in nature to a considerable degree, but the dissimilarity of the blood forms and Futaki's cultural forms is too marked to be explained on this basis. With regard to the forms found in tissues, careful search has been made in the organs of animals infected with the present strain, which have been killed at various stages of the infection and also during convalescence, without finding any spiral organism other than the form as described in the peripheral blood. This is in agreement with the findings of Row and other workers. In the present case, in attempting to cultivate the parasite on Shimamine's medium, numerous filaments were found, which closely simulated the appearances of *Leptospira* and of *Spironemata*. It therefore seems probable that Kaneko and Okuda have been dealing with some spirochaetal infection superimposed on that of the rat-bite fever organism, and that Futaki and his co-workers have either cultivated some other organism, or have mistaken the filamentous threads in the medium for true spirochaetes. The work of Thomson (1923) on pseudo-spirochaetes may have some bearing on this question.

The conclusion must be arrived at that the rat-bite fever organism with its terminal flagella, and non-flexible, non-undulating, spiral body should be referred at present to the genus *Spirillum*, Ehrenberg, 1830.

In the consideration of the specific name, which should be applied to the rat-bite fever organism, the first point to be decided is whether more than one distinct species of *Spirillum* has been described in the blood of mice and rats, bandicoots, etc., and if not, whether this is the same as that causing rat-bite fever. Firstly, as regards the *Spirilla* in the blood of mice, rats, etc., the prior discovery of such an organism rests with Carter. That Carter was dealing with a true *Spirillum* seems clear from his description of the morphology and of the characteristic movements. Following Carter, the *Spirochaeta muris* of Wenyon, with which can be grouped the *Spirochaeta laverani* of Breinl and Kinghorn as it came from the same source, was the next to be given a specific name. That this was a *Spirillum* also there is now no doubt, and so far as its morphology is concerned, its variations from *Spirillum minor* are such as may be seen in different strains of the same species. Carter was unable to demonstrate his organism in an experimentally infected rat and monkey, but this is quite in accordance with the findings of later workers using strains of the rat-bite parasite. It is quite impossible to prove that Wenyon, and the large number of workers such as Lingard, Borrel, Deetjen, etc., who found similar spiral organisms in mice and other animals, but did not name them, were not dealing with distinct species, but the contention is that the bulk of evidence, in view of more recent work, is so strongly suggestive as almost to amount to a certainty that they were describing *Spirillum minor*. Admittedly slight differences do occur, in morphology and also in the action on inoculated animals, but similar differences are found in undoubted rat-bite fever strains, and it is suggested that these variations are insufficient to justify the bestowal of new generic and specific names.

The question next arises as to whether the organism isolated by Futaki and his associates is the same as those described in the blood of mice, etc., by the previous workers. In parenthesis it may be said that the *Spirochaeta petit* of Row, in view of the work of Parmanand, is here considered to be the same as the *S. morsus muris* of the Japanese workers, and that, therefore, only one species of *Spirillum* is known to cause true rat-bite fever in man.

The problem resolves itself to this : does there exist in the blood of mice, rats, cats, etc., a *Spirillum* which can be distinguished from other *Spirilla* in the same animals, only by its transmissability to

man ? This, needless to say, is incapable of absolute proof either way, but the submission is that the preponderance of evidence points to the non-existence of a distinct ' human ' species.

1. As regards morphology the forms isolated in human strains cannot be differentiated from the forms in animals naturally infected.

2. Human strains differ from each other in their inoculability into, and reaction in experimental animals. Thus one strain may be highly virulent in guinea-pigs, and show parasites in the animals' blood, whereas another may cause some temporary discomfort only. One strain may be easily inoculable into rats and another may not. Such differences as do occur between human and animal strains in their inoculability and reactions are no greater than may be found in two divergent human strains.

3. It has been proved that serum from patients recovering from rat-bite fever, and also cat-bite disease, contains immune bodies which will immobilise *Spirilla* isolated from naturally infected wild rats.

The conclusion arrived at is that the *Spirillum*, which causes rat-bite fever in man, is the same as the one which occurs naturally in the blood of animals. Following the rule of priority, therefore, the name for the causal organism should be *Spirillum minor*, Carter, 1887. Unfortunately, in naming his organism, Carter did not take into account the fact that the generic name *Spirillum* is neuter. When such a mistake in gender has been made, it is permissible, under the International Rules of Botanical Nomenclature (Art. 57), to make the necessary correction, while the corrected name is cited from the original author and publication. *Spirillum minor*, therefore, becomes *Spirillum minus*, Carter, 1887.

## CONCLUSION

1. Up to the present there is no reason for supposing that the *Spirilla* in the blood of naturally infected mice, rats, bandicoots, etc., belong to more than one species.

2. The species of *Spirillum*, which is the causal organism of rat-bite fever in man, is not distinct from the species found naturally in the blood of animals.

3. The correct name for the causal organism of rat-bite fever in man is

*Spirillum minus*, Carter, 1887.

Synonyms :

*Spirochaeta laverani*, Breinl and Kinghorn, 1906.

*Spirochaeta muris*, Wenyon, 1906.

*Spirochaeta morsus muris*, Futaki, Takaki, Taniguchi and Osumi, 1917.

*Leptospira morsus muris* (Futaki, Takaki, Taniguchi and Osumi, 1917) emend. Manson-Bahr, 1922.

*Spirochaeta petit*, Row, 1922.

*Treponemella muris* (Wenyon, 1906), emend. Sangiorgi, 1922.

*Spironema minor* (Carter, 1887), emend. Sangiorgi, 1922.

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# NOTES ON CERTAIN CESTODES IN THE SCHOOL OF TROPICAL MEDICINE, LIVERPOOL

BY

T. SOUTHWELL

(*Received for publication* 21 *April*, 1924)

Order PSEUDOPHYLLIDEA, Lühe, 1899.
Family *DIBOTHRIOCEPHALIDAE*, Lühe, 1902.
Sub-Family *DIBOTHRIOCEPHALINAE*, Lühe, 1899.

*Dibothriocephalus decipiens* (Diesing, 1850), Lühe, 1899. One specimen and a fragment from a leopard (*Felis pardus*) Manáos, Amazonas. Collected and presented by Dr. R. M. Gordon. July 10, 1921.

*Duthiersia fimbriata* (Diesing, 1850), Montic. and Crety, 1891. Several specimens from a monitor Lizard, *Varanus* sp. Madras, India. April 1, 1905.

Order CYCLOPHYLLIDEA, Lühe, 1910.
Family *ANOPLOCEPHALIDAE*, Fuhrmann, 1907.
Sub-family I. *ANOPLOCEPHALINAE*, R. Blanchard, 1891.

*Bertiella studeri* (R. Bl. 1891) Stiles and Hassall, 1902. One specimen measuring 150 mm. in length, 13 mm. in breadth, and 2 mm. in thickness, from a monkey *Cercopithecus pygerythrus*. Ngoa, North-eastern Rhodesia. July 14, 1912.

Collected and presented by Professor Warrington Yorke, M.D.

The species differs from *B. cercopitheci*, Beddard, 1911, in that the eggs bear a pyriform apparatus, and the pores are regularly alternate.

*Bertiella cercopitheci*, Beddard, 1911. One immature specimen and a fragment, from *Cercopithecus pygerythrus*, Ngoa, North-eastern Rhodesia, collected and presented by Professor Warrington Yorke, M.D.

Sub-family II. *LINSTOWINAE*, Fuhr., 1907.

*Oochoristica truncata* (Krabbe, 1879) Zschokke, 1905. Very numerous specimens from the intestine of *Agama colonorum*, Accra, Gold Coast. Collected and presented by Dr. J. W. Scott Macfie, 1912.

*Linstowia ameivae*, Beddard, 1914. Two fragmented specimens from *Ameivia dorsalis*, Kingston, Jamaica. Collected and presented by Professor R. Newstead, F.R.S.

The specimens agree closely with Beddard's description except in the number of testes.

Beddard states that

'The *testes* lie posteriorly to the vitelline gland, and reach forward on either side of it; they do not, however, extend laterally of the ovary. In a given segment the testes were visible in 18 consecutive sections. The largest number counted in the middle of the series was 43. I therefore calculate the total number to be about 200. The testes do not extend laterally beyond the lateral water-vascular vessels.'

In the specimens from Kingston, about 40 testes, only, are present in each segment, but they are very large and would accordingly be present in many consecutive sections.

There can be little doubt that the genera *Linstowia*, Zschokke, 1898, and *Oochoristica*, Lühe, 1898, are very closely related, if not identical.

Family *DAVAINEIDAE*, Fuhr., 1907.

Sub-family *DAVAINEINAE*, Braun, 1900.

*Davainea microscolecina*, Fuhrmann, 1909. Several specimens of this species collected and presented by Professor Warrington Yorke, M.D., Nawalia, North-eastern Rhodesia. June 28, 1911.

Host :—An unknown bird, the vernacular name of which is *Nduwaruwo*.

Family *HYMENOLEPIDIDAE*, Railliet and Henry, 1909.

Sub-family *HYMENOLEPIDINAE*, Ransom, 1909.

*Hymenolepis interruptus*, Clerc, 1906. Two specimens from a sparrow *Passer domesticus*, Hoylake, Cheshire, England. Collected and presented by Professor J. W. W. Stephens, F.R.S.

*Echinocotyle nitidulans* (Krabbe, 1882), Fuhrmann, 1906 (figs. 1-3). Numerous specimens from the intestine of *Tringa alpina*, Hoylake, Cheshire, England.

As the anatomy of this worm is not known, the following details are added.

The worms measure about 7 mm. in length and the maximum breadth is about 140 $\mu$; the number of segments varies from about 70 to 112, the average number in nine worms being 93. The segments are imbricated, and the last ones are almost square and measure about 100 $\mu$. The head measures about 130 $\mu$ in length and 145 $\mu$ in breadth. The rostellum is very prominent and measures about 75 $\mu$ in length, terminating anteriorly in an expansion. It can be withdrawn into a very deep muscular sac which is dilated and extends into the neck region. It is armed with a single crown of ten simple hooks which measure 55 $\mu$. The neck measures about 380 $\mu$ in length. There are three testes all in a line, and situated posteriorly.

Fig. 1. *Echinocotyle nitidulans.* Head. × 160.

The cirrus pouch is enormous and very muscular; it extends practically across the entire segment and has a breadth of about 25 $\mu$. No spines could be seen on the cirrus even under an oil immersion lens. The vas deferens on leaving the cirrus pouch, runs parallel, and posterior to the pouch, dilating into a seminal vesicle. Immediately median to this vesicle a prominent prostate gland can be seen enveloping a short portion of the vas deferens; it appears to be chitinous and resembles a spine; this portion of the vas deferens is very striking under high power magnifications. The vas deferens then splits up into the vasa efferentia.

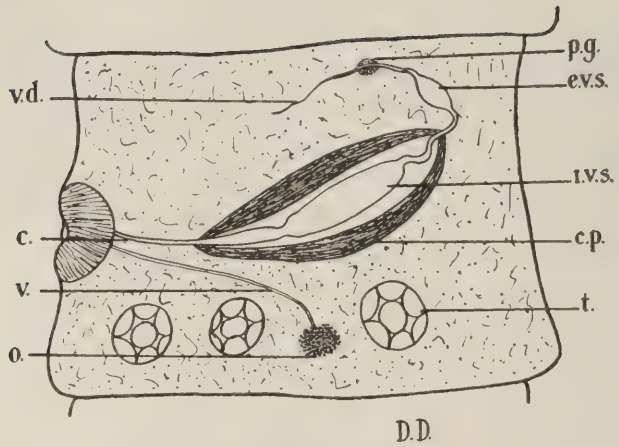


FIG. 2. *Echinocotyle nitidulans.* Segment nearly ripe. *v.d.*—vas deferens; *c*—cirrus; *v*—vagina; *o*—ovary; *t*—testes; *c.p.*—cirrus pouch; *i.v.s.*—internal seminal vesicle; *e.v.s.*—external seminal vesicle; *p.g.*—prostate gland. × 320.

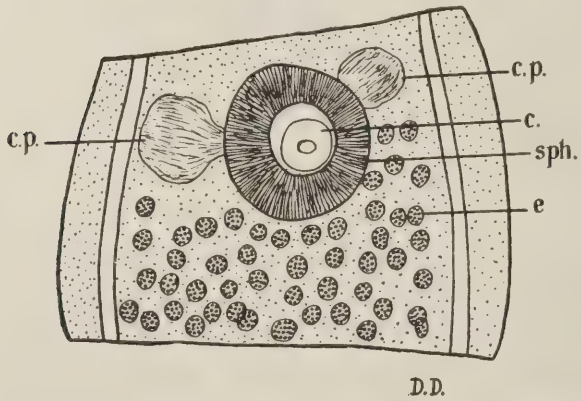


FIG. 3. *Echinocotyle nitidulans.* Side view of gravid segment showing pore. *c.p.*—cirrus pouch; *e*—eggs; *sph.*—sphincter muscle; *c*—cirrus. × 320.

The genital pores are unilateral and are surrounded by a very powerful sphincter muscle.

The uterus is a simple sac; in many segments it was full of immature eggs. No ripe eggs were seen.

There appears to be no reason for separating this genus (which contains eight species only) from the genus *Hymenolepis*.

Family *TAENIIDAE*, Ludwig, 1886.
Sub-family *TAENIINAE*, Stiles, 1886.

*Multiceps serialis* (Gervais, 1847), Stiles and Stevenson, 1905. Four specimens from a Hyena (spotted leopard), Ngoa, North-eastern Rhodesia. Collected and presented by Professor Warrington Yorke, M.D.

The worms measured about 45 cms. in length and the last segments about 1·3 cms. in length.—Genital pores large, and situated behind the middle of the lateral margin.

Head with a double crown of hooks, the larger measuring 160 $\mu$ in length and the smaller 85 $\mu$ in length. Uterus with from 14 to 21 lateral branches.

This parasite has hitherto been recorded from *Canis familiaris*, *Lepus caniculus* and *Sciurus carolinensis*.

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# A SURVEY OF THE PARASITES FOUND IN NATIVES OF SIERRA LEONE

BY

P. A. MAPLESTONE

(*From the Sir Alfred Jones Research Laboratory, Freetown, Sierra Leone*)

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This investigation was carried out with the object of obtaining an indication of the infection rate among Sierra Leone natives of the common intestinal parasites.

The stools of five hundred natives, all prisoners in the Freetown gaol and all adult males, were examined. The results are, therefore, not quite representative of the native population as a whole, as women and children are not included. Nevertheless, so far as it goes, it is fairly comprehensive, because all the tribes found in Sierra Leone Protectorate are represented and 75 per cent. of the people examined were living in Freetown at the time of arrest ; the remaining 25 per cent. were living in various parts of the Protectorate. Gaol infection may be excluded, for the examinations were made within a few days of incarceration in the prison.

Only a single specimen of faeces was examined from each individual, but at least two smears in normal saline under a seven-eighths inch square cover glass were completely examined in every case, and where necessary, specimens in Lugol's solution were made as well. In addition to this examination, a portion of the faeces was mixed in saturated salt solution for detection of helminth eggs that had been missed in the thin smears.

## (*a*) Protozoa

Table I gives a list of all the Protozoa encountered, with the number of infections expressed in percentages.

Table I

| Parasite | Per cent. infected | Parasite | Per cent. infected |
|---|---|---|---|
| *Entamoeba histolytica* ... ... | 15 | *Giardia intestinalis* ... ... | 2·2 |
| ,, *coli* ... ... ... | 43·6 | *Chilomastix mesnili* ... ... | 1·8 |
| *Endolimax nana* ... ... ... | 9·8 | *Trichomonas hominis* ... ... | 2·2 |
| *Iodamoeba butschlii* ... ... | 13 | *Enteromonas intestinalis* ... ... | 2·4 |
| | | Coprozoic flagellates ... ... | 14·6 |

The nomenclature in the above table is taken from Dobell and O'Connor (1921), and under 'Coprozoic flagellates' are grouped all the species so classified by the same authors, because their cysts are not distinguishable from each other in fresh smears.

There is nothing remarkable in the above figures, except perhaps the small number of natives infected with certain flagellates, especially *G. intestinalis*. Vegetative forms of the Amoebae and their cysts have been included under the one head. Five cases of infection of Amoebae with *Sphaerita* spp. were encountered; two of these were in *E. histolytica*, two in *E. coli* and one in *I. butschlii*.

There were, of course, several cases of infection with two or more parasites, but no analysis of these has been made, for it is not considered that such figures are of any practical importance.

## (*b*) Helminths.

In Table II are given a list of the intestinal worms found, the diagnosis being made by the presence of eggs in the faeces. The infection rate is expressed in percentages of the 500 stools examined.

TABLE II

| Parasite | Per cent. infected | Parasite | Per cent. infected |
|---|---|---|---|
| *Ascaris lumbricoides* ... ... | 36·6 | *Enterobius vermicularis* ... ... | 1·6 |
| *Trichuris trichiura*... ... ... | 18·6 | Hookworm* ... ... ... | 76·6 |
| *Strongyloides stercoralis* ... ... | 15·2 | *Taenia* sp. ... ... ... | 3·2 |

* Dr. Adler, of the Sir A. L. Jones Laboratory, examined 4,305 adult Hookworms taken from 47 different cases and found that 91·3 per cent. were *N. americanus* and 8·7 per cent. were *A. duodenale.* Of the total of 376 *A. duodenale*, 302 came from two cases who were not gaol prisoners. Worms examined from prisoners were *N. americanus* 98 per cent. and *A. duodenale* 2 per cent., which is probably the more correct figure for the Colony as a whole.

The percentage of *S. stercoralis* is probably much lower than the actual number, for many faeces, negative by ordinary examination, were found to contain this parasite after culturing which was being done for another purpose. Indeed, it is considered probable that if this method were employed, the infection rate with *S. stercoralis* would be found to be almost, if not quite, as high as that with Hookworm.

The *Taenia* cases are probably all *T. saginata*, for that is the only cestode the writer has found in natives in Freetown ; the eggs were slightly oval and none were round, this being given as a diagnostic character between the eggs of *T. saginata* and *T. solium.*

### MICROFILARIA.

Thick films of night blood were examined in 288 cases and *Mf. bancrofti* were present in 16·3 per cent., and *Mf. perstans* in 2·4 per cent. Out of the 195 thick films of day blood no infections with *Mf. Loa* were discovered.

### (*c*) A NOTE ON THE USE OF SATURATED SALT SOLUTION FOR THE DETECTION OF HELMINTH EGGS IN FAECES.

Lane (1922) has discussed this method in detail along with others, and he drew attention to its value for the detection of eggs other than those of hookworm, so it is only proposed to deal with the method quite briefly, in confirmation of Lane's remarks.

*Technique :—*

Small solid watch glasses were used for mixing the faeces; they contain about 8 c.c. when filled sufficiently to have a distinct convex meniscus. Care was taken to use a little less than 0·5 grams of faeces so as to keep within the limit of effective concentration; the importance of this precaution has been shown by Lane. It was found quite easy after a little practice to take the right amount of faeces by guesswork. The faeces were first thoroughly emulsified in about 2 c.c. of salt solution by stirring with a glass rod, more salt solution was then added, until there was a distinct convex meniscus above the surface of the watch glass. The emulsion was left for about five minutes to allow the eggs to come to the surface, and a glass slide 3 ins. by 2 ins. was laid on top of it. The slide was lifted off in about half a minute and rapidly turned over to bring the wet surface uppermost without allowing the fluid to run off, and it was examined under the low power of the microscope without a cover slip. The eggs, whatever the species, were always floating on the surface of the film of fluid, therefore even in the case of hookworm eggs, the character they possess of adhering to glass played no part in the success of the method.

The saturated salt solution was always prepared by boiling excess of salt in water and allowing it to cool, and as Lane (1922) has pointed out, this method of its preparation always assured the obtaining of a solution of slightly over 1,200 specific gravity.

Table III gives the number of times each species of egg was found in plain smears and the number of times it was found in salt solution after having been missed in plain smears; when eggs were found in plain smears, they were always found again by salt concentration.

Table III

| | *Ascaris* | | *Trichuris* | | *Strongyloides* | | *Oxyuris* | | Hookworm | | *Taenia* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Plain smear ... | 159 | 86·9 | 36 | 38·7 | 74 | 97·4 | 6 | 75 | 223 | 58·2 | 15 | 88·2 |
| Salt solution ... | 24 | 13·1 | 57 | 61·3 | 2 | 2·6 | 2 | 25 | 160 | 41·8 | 2 | 11·8 |

The different percentages of positive results for different eggs is due to the number present in a given volume of faeces; those

species such as *Ascaris*, in which a large number of eggs are present give a relatively high number of positives in plain smears and a correspondingly low number after salt concentration, whereas in the case of *Trichuris* which only has few eggs present, the reverse is the case. As Lane has already found, the larvae of *Strongyloides* do not readily come to the surface in salt solution, in fact, many cases which were positive in plain smears were negative in salt concentration.

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# A CRITICAL EXAMINATION OF STOLL'S METHOD OF COUNTING HOOKWORM EGGS IN FAECES

BY

P. A. MAPLESTONE

(*From the Sir Alfred Jones Research Laboratory, Freetown, Sierra Leone*)

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Stoll (1923) published a method of estimating the number of hookworm eggs in faeces. The evidence he adduced of the accuracy of his method may be summarised as follows :—

(1) No cultures of the same faeces produced as many larvae as were shown by egg counts.

(2) Soft stools gave less eggs than solid faeces from the same individual.

(3) The first portion passed of a formed stool gave higher counts than random samples of the whole of the same stool. This is explained by the fact that the first portion of a formed stool is drier and hence the eggs are more concentrated in that portion than in later and softer parts of the same stool.

(4) Random samples from the last and softer part of a stool gave slightly lower egg counts than the whole stool mixed.

(5) Counts from day to day from a single individual remained uniform, and this also applied to counts of *Ascaris*, *Trichuris*, and *Schistosomum* eggs.

(6) Counts from two distinct samples of the same stool are always fairly close together.

Stoll's evidence seems conclusive, but it is at best only circumstantial and the writer found it frequently inaccurate in one particular, viz. : that control cultures always produced fewer larvae than the number of eggs counted in the same faeces. This led to examining the method more closely and testing it in various ways.

Faeces were taken and counts performed in exactly the same way as Stoll describes, and, in addition, small portions (about 200 milligrammes) of the same faeces were accurately weighed in small solid watch glasses and thoroughly mixed with saturated salt solution. Slide after slide was then applied to the surface of the fluid in the watch glasses and all the eggs so obtained counted. This was continued until it was thought that nearly all the eggs had been removed.

Specimen 1.

Counted by Stoll's method. 1st Count, 8. 2nd Count, 10. No. of eggs per gram of faeces average 900.

219 mgr. of faeces mixed with saturated salt solution gave 369+333+162+76 +103+7 eggs on six successive slides.

Total 1,050, which = 4,794 eggs per gram.

202 mgr. of faeces mixed with sat. salt sol. gave 352 + 274 + 251 + 148 + 66 eggs on five successive slides.

Total 1,091, which = 5,391 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 5,092.

Control cultures produced an average of 4,080 larvae per gram.

Specimen 2.

Counted by Stoll's method. 1st Count, 6. 2nd Count, 6. No. of eggs per gram of faeces average 600.

201 mgr. of faeces mixed with sat. salt sol. gave 9 + 19 + 16 + 11 eggs on four successive slides.

Total 50, which = 274 eggs per gram.

201 mgr. of faeces mixed with sat. salt sol. gave 15 + 9 + 14 + 2 eggs on four successive slides.

Total 40, which = 199 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 236.5.

Control cultures were not done.

Specimen 3.

Counted by Stoll's method. 1st Count, 2. 2nd Count, 1.

No. of eggs per gram of faeces average 150.

201 mgr. of faeces mixed with sat. salt sol. gave 8 + 9 + 11 + 6 + 10 + 3 eggs on six successive slides.

Total 47, which = 233 eggs per gram.

200 mgr. of faeces mixed with sat. salt sol. gave 13 + 13 + 5 + 9 + 3 + 3 eggs on six successive slides.

Total 46, which = 230 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 231.5.

Control cultures produced an average of 353 larvae per gram.

Specimen 4.

Counted by Stoll's method. 1st Count, 2. 2nd Count, 1.

No. of eggs per gram of faeces average 150.

200.5 mgr. of faeces mixed with sat. salt sol. gave 8 + 35 + 21 + 7 + 3 eggs on five successive slides.

Total 74, which = 369 eggs per gram.

200.5 mgr. of faeces mixed with sat. salt sol. gave 18 + 15 + 32 + 13 + 8 eggs on five successive slides.

Total 86, which = 429 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 369.

Control cultures produced an average of 450 larvae per gram.

Specimen 5.

Counted by Stoll's method. 1st Count, 6. 2nd Count, 6.

No. of eggs per gram of faeces average 600.

207 mgr. of faeces mixed with sat. salt sol. gave 27 + 52 + 3 + 2 + 0 on five successive slides.

Total 84, which = 406 eggs per gram.

201 mgr. of faeces mixed with sat. salt sol. gave 24 + 35 + 16 + 2 + 0 on five successive slides.

Total 77, which = 383 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 394.5.

Control cultures produced an average of 796 larvae per gram.

Specimen 6.

Counted by Stoll's method. 1st Count, 100. 2nd Count, 100.

No. of eggs per gram of faeces average 10,000.

210 mgr. of faeces mixed with sat. salt sol. gave 323 + 319 + 471 + 162 + 72 on five successive slides.

Total 1,347, which = 6,414 eggs per gram.

Control cultures produced an average of 8,833 larvae per gram.

Stoll's method was further checked in the following manner :— Two counts were made by Stoll's method, his instructions being followed in every detail. About ten grams of faeces were placed in a beaker and balanced against an equal weight of water, the two being thoroughly mixed by stirring until the mixture was perfectly homogeneous. Three-gram lots of this mixture were now weighed out

and counted by Stoll's method. It was previously found that faeces are practically of a specific gravity of 1, therefore when mixed with an equal weight of water, a given weight of the mixture will only contain half the amount of faeces that is contained in the same weight of undiluted faeces. In Stoll's method, using undiluted faeces, the number obtained by counting is multiplied by 100 to ascertain the number of eggs in a gram of faeces ; accordingly, if the same weight of the diluted faeces is taken and counted by Stoll's method, it will be necessary to multiply the count by 200 to obtain

TABLE I

SUMMARY OF ABOVE COUNTS

| Number of specimen | Eggs per gram by Stoll's method | Eggs per gram by salt concentration | Larvae per gram in control cultures |
|---|---|---|---|
| 1 | 900 | 5,092 | 4,080 |
| 2 | 600 | 236·5 | Not done |
| 3 | 150 | 231·5 | 353 |
| 4 | 150 | 369 | 450 |
| 5 | 600 | 394·5 | 796 |
| 6 | 10,000 | 6,414 | 8,833 |

the number of eggs per gram in the undiluted faeces, and if Stoll's method is correct these two results should be approximately the same. If the dilution is still more increased by taking part of the faeces and water mixture already made and mixing it with an equal weight of water, a count of this dilution, if counted by Stoll's method, will have to be multiplied by 400 to give the number of eggs per gram in the original faeces. Further dilution in the same proportions will require multiplication by 800.

The above process was carried out with several specimens of faeces and the results are given in Table II. In this table 'first dilution' means original faeces plus an equal weight of water, 'second dilution' means part of 'first dilution' plus an equal weight of water, and 'third dilution' means a part of 'second dilution' plus an equal weight of water.

Table II

| Character of Faeces | Undiluted Faeces | | | First Dilution | | | Second Dilution | | | Third Dilution | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st Count | 2nd Count | Eggs per gram | 1st Count | 2nd Count | Eggs per gram | 1st Count | 2nd Count | Eggs per gram | 1st Count | 2nd Count | Eggs per gram |
| Soft, formed... ... | 100 | 117 | 10,850 | 69 | 76 | 14,500 | 51 | 41 | 18,400 | — | — | — |
| | — | — | — | 78 | 72 | 15,000 | — | — | — | — | — | — |
| Very watery... ... | 23 | 17 | 2,000 | 9 | 8 | 1,700 | — | — | — | — | — | — |
| | 21 | 20 | 2,050 | 12 | 12 | 2,400 | — | — | — | — | — | — |
| Soft, formed... ... | 5 | 4 | 450 | 8 | 4 | 1,200 | 2 | 2 | 800 | — | — | — |
| Liquid ... ... | 26 | 41 | 3,350 | 15 | 22 | 3,700 | 12 | 9 | 4,200 | — | — | — |
| Soft, formed... ... | 53 | 65 | 5,900 | 95 | 102 | 19,700 | 56 | 64 | 24,000 | — | — | — |
| | 56 | 65 | 6,050 | 86 | 95 | 18,100 | 41 | 50 | 18,200 | — | — | — |
| Watery, with mucus | 9 | 6 | 750 | 3 | 0 | 300 | 1 | 0 | 200 | — | — | — |
| Formed ... ... | 0 | 0 | 0 | 1 | 1 | 200 | 0 | 1 | 200 | — | — | — |
| Formed, a little softer than 7 | 1 | 1 | 100 | 0 | 3 | 300 | 0 | 3 | 600 | 0 | 0 | 0 |
| Soft, unformed ... | 0 | 1 | 50 | 0 | 1 | 100 | 1 | 1 | 400 | — | — | — |
| Liquid ... ... | 2 | 2 | 200 | 2 | 4 | 600 | 3 | 2 | 1,000 | 2 | 1 | 1,200 |
| Liquid ... ... | 11 | 6 | 850 | 7 | 3 | 1,000 | 0 | 2 | 400 | 3 | 2 | 2,000 |

All the weighings and counts were done personally by the writer with the greatest possible care, and accuracy was in no case sacrificed on account of the time taken; slides were counted twice if there was any doubt that eggs had been missed or counted more than once owing to inaccurate moving of the mechanical stage of the microscope.

The above results obtained by checking Stoll's method against salt concentration and dilution of faeces indicate that the method is not accurate within ten per cent., which is the claim of the originator.

Examination of Table II suggests that the number of eggs obtained by this method varies with the consistency of the original specimen of faeces, higher counts being obtained when the faeces are liquid. The reason for this is difficult to explain, for shaking with glass beads in a solution of deci-normal NaOH for a minute or more, as recommended by Stoll, seems to cause just as complete comminution of the faeces whether they are solid, soft, or liquid to begin with.

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# ON THE LIFE HISTORY OF A REPTILIAN TAPEWORM *(SPARGANUM REPTANS)*

BY

F. J. MEGGITT, M.Sc., Ph.D.

PROFESSOR OF BIOLOGY, UNIVERSITY OF RANGOON

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Plate IX

The genus *Sparganum* was first proposed by Diesing (1854, 573) for those Pseudophyllidean cestodes the adult stages of which were unknown, and has included at various times *S. affine*, Diesing, 1854, *S. ardeae-coeruleae* (Diesing, 1850), *S. baxteri*, Sambon, 1907, *S. ellipticum*, Molin, 1858, *S. erinacei-europaei* (Rudolphi, 1819), *S. falconis* (Rudolphi, 1819), *S. lanceolatum*, Molin, 1859, *S. lanii-pomerani* (Rudolphi, 1819), *S. mansoni* (Cobbold, 1883), *S. mygales-moschatae* (Rudolphi, 1819), *S. proliferum* (Ijima, 1905), *S. raillieti*, Rátz, 1913, *S. reptans* (Diesing, 1850), *S. sebago*, Ward, 1910, *S. strigis-accipitrinae* (Rudolphi, 1819). Most of these are probably synonyms, but the absence of diagnostic characters renders identification impossible except by feeding experiments. Of the above fifteen forms, only the life-histories of *S. mansoni* and *S. raillieti* have been elucidated.

*S. reptans* is essentially parasitic in reptiles, occurring in the connective tissue and between the muscles—usually dorsal—of *Amphisbaena flavescens**, *Anabates lucinioides*, *Drymobius bifossatus* (Raddi, 1820), *Elaps marcgravii*, Wied, 1820, *Erythrolampus aesculapii* (L.1754) *Herpetodryas carinata* (L.1754), *Lachesis lanceolatus* (Jonnès, 1816), *L. neuwiedii* (Wagl. 1824), *Leptophis liocerus* (Wied, 1824) *Oxyrhopus cloelia* (Daud, 1803), *Pseudophis*

* In consequence of the scanty literature at my disposal, it has proved impossible to check all the host names.

*bivittatus*, *P. cinerascens* and *Xenodon merremii* (Wagl. 1824). It has also been recorded from :—

AMPHIBIA : *Alcedo americana*, *Hydroscopus plumbeus*, *Lyophis regius*, *Rhynaspis proboscidea*, *Spilotes pullatus*.

AVES : *Ardea coccoi*, *A. leuce*, *A. lineata*, *Corvus azureus*, *C. cristalellatus*, *C. cyanomelas*, *C. pileatus*, *Ibis albicollis*, *Merganser brasiliensis* (Vieill.), *Merula albiventer*, Spix, *M. rufiventris*, Vieill., *Molothrus ater* (Bodd.), *Musicapa psalura*, *Nonnula rubecula* (Spix), *Nothocrax urumutum* (Spix), *Ostinops decumanus* (Pallas), *Pandion haliaëtus* (L.) *Rhamphastos temminckii R. toco* (Müller), *Rhynchotus rufescens* (Temm.), *Strix grallaria*, *Tantalus loculator* (L.).

MAMMALIA : *Canis azarae*, Wied, 1826, *Chloroceryle americana*, *Chrysothrix sciurea* (L.1766), *Didelphys brachyura*, *D. opossum*, Seba, 1734, *Felis mitis*, Cuvier, 1820, *F. pardus*, L.1766, *F. tigrina*, Erxleben, 1777, *Galera barbara* (L.1766), *Gulo barbatus*, *Hapale melanura* (Geoffroy, 1812), *Holochilus brasiliensis* (Geoffroy, 1819), *Lutra brasiliensis*, Zimmermann, 1780, *L. paranensis*, Rengg, 1830, *Nasua narica* (L.1766), *Noctilio leporinus*, L.1766, *Saïmiris sciurea* (L.1766).

All records, however, other than those from reptiles, should be regarded with suspicion, the absence of distinguishing characters rendering it probable that several species have been confused under one name.

*S. reptans* is an exceedingly common parasite in Burmese snakes. From one *Tropidonotus*, sp., twenty specimens were obtained, from another two, and from four more approximately fifteen each. With this material an attempt was made to discover the life-history. On January 20th, ten active specimens were given to a puppy a month old, and six to a human volunteer. On February 20th a further ten were given to the dog, who in the meantime had grown and fattened. He was killed on March 3rd, and was found to contain three adult *Dipyllidium caninum*, three full-grown *Diphyllobothrium* and two scoleces of the same genus. The experiment is not conclusive as no time was available for previous faecal examinations of the animal nor for treatment with anthelminthics. Considering the youth of the dog and the absence of any records of Pseudophyllidea from Indian dogs, it is exceedingly improbable that the

*Diphyllobothrium* were present previous to the experiment. The presence of the worms produced in the animal no symptoms whatever. No trace of infection has been found in the faecal examinations of the human subject with the exception of a single egg, probably due to a contaminated slide. Attempts were made to ascertain if the life-history conforms to the type of *D. latum* and *D. mansoni* described by Rosen (1918) and Okomura (1919) respectively, but up to the present no procercoids have been found in the experimental Entomostraca.

## PLEROCERCOID STAGE

The plerocercoid stage is usually to be found in small sacs on each side of the vertebral column between the skin and musculature of the dorsal surface, also amongst the connective tissue. It is motionless *in situ*, but upon being deposited in warm water becomes exceedingly active, wriggling in a manner reminiscent of a nematode. The scolex in particular performs active and regular movements. The tip is first protruded like a small tongue, becomes flattened while the sides swell up to form a truncated square pyramid, and is then retracted again, leaving a small pit at the apex of the pyramid. The whole anterior extremity subsides immediately, to repeat the same movements without pause. There is no definite scolex nor bothridia, only the mobile anterior extremity. The alleged terminal invagination is the result of contraction consequent upon fixation and, while probably performing the functions, has no trace of the structure of a sucker. External and internal segmentation is usually absent, but may occasionally in unusually long forms be represented by a few posterior transverse striations. The body is a slender ribbon with an anterior globular swelling, varying in length from 2 to 100 mm. and is capable of asexual reproduction by fragmentation but not by proliferation. In several cases strobilae were observed which were obviously the result of fragmentation and which were leading an independent existence. The internal anatomy showed nothing of note except the absence of 'nutritive bodies' described by Ijima (1905, 15) and myself (1924, 53) for *S. prolifer* and *Sparganum*, sp. respectively. In the absence of other distinctions it is possible that this character may be of use in identification.

## ADULT FORM

Length 1000 mm. by 9 mm. wide, clearly segmented externally though with but little overlapping of the proglottides. Scolex (Pl. IX, fig. 1) elongated, 800$\mu$ long by 40$\mu$ wide, bearing two long shallow bothridia with indistinct edges, merging anteriorly and posteriorly into the scolex. Neck elongated. All segments broader than long. Musculature weak, consisting of a narrow and feeble layer of longitudinal muscles: transverse muscles could not be seen. Excretory vessels indistinct, consisting of four to eight longitudinal trunks on each side of the proglottis, connected by an extensive and complicated capillary system. Genital pores (Pl. IX, fig. 2) superficial, on same surface of proglottis. Male in anterior sixth, central: vaginal posterior to it and slightly lateral: uterine more posterior and central. Cirrus-sac extending half-way to opposite surface, external vesicula seminalis nearly reaching aporal cortical parenchyma. Testes in transverse sections 8 to 10 each side, in longitudinal vertical ones 9 to 11, total 144 to 220: in two separate lateral bands, slightly converging anteriorly. Ovary bi-lobed, reticulate. Shell-gland large, at posterior margin of proglottis. Vitelline glands lateral, converging and meeting anteriorly, and leaving free a central space one-twelfth to one-seventh of width of proglottis.

Eggs 53$\mu$ to 59$\mu$ by 36$\mu$ to 40$\mu$, operculated: immature when deposited, onchosphere develops while egg lies in water.

From the characters just given, it may be seen that the adult of *S. reptans* belongs to the genus *Diphyllobothrium*. Altogether forty-seven species of this genus have been recorded, but the descriptions of only twenty-eight contain characters of diagnostic value, the remainder being concerned only with length and breadth of scolex and strobilus, shape of proglottides, etc.

An examination of the following table giving the species of this genus, indicates that well-marked features (distribution of testes, position and number of uterine coils, etc.) separate *D. reptans* from the majority of forms. From the remainder many may be separated by the size of the egg, a character liable to variation but only within fairly definite limits. *D. exile* and *D. fissiceps* have only been recorded once and are dubious species, while *D. nasuta* according to Fuhrmann

(1908, 95) should no longer be recognised. Minor differences separate *D. stemmacephalum* (shape of bothridia), *D. folium* (absence of neck and shape of proglottides, *D. rufum* (pigmentation of scolex) and *D. americanum* (absence of neck). These features are of slight value in themselves, but constitute the only distinguishing marks of the species. From *D. strigis-accipitrinae*, *D. decipiens*, *D. sulcatum*, *D. similis*, and *D. marginatus* the present form cannot be separated at all, owing to the absence of any description. The description of *D. clavatum* is not accessible here. It may, therefore, be concluded with reasonable accuracy, that *D. reptans* is a distinct species. The adult host remains to be discovered. The dog is obviously only an experimental host, the true one is probably a carnivore or an avian scavenger.

Specimens of adult and larval forms have been deposited in the Molteno Institute for Parasitology, Cambridge.

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| Species | | Genital pore | Uterine pore | Cirrus-sac | Testes | Uterine coils | Enlargement of uterus | Eggs (in $\mu$) | Host |
|---|---|---|---|---|---|---|---|---|---|
| *D. americanum* ... | Hall and Wigdor, 1918 | ... | ... | ... | ... | ... | ... | ... | *Canis familiaris* |
| *D. archeri* ... ... | (Leiper and Atkinson, 1914) | Half-way | Median | 5 lobed internally | Scattered | ... | ... | ... | *Ogmorhinus weddelli* |
| *D. canadense* ... | Cooper, 1921 | Anterior third, vaginal lateral | Median | ... | 150, 2 lateral fields joined anteriorly | 8 to 10 each side | Absent, coils break down into sac | 56 to 59 by 37 to 39 | *Corvus corax principalis* |
| *D. clavatum* ... | Railliet and Henry 1912 | ... | ... | ... | ... | ... | ... | ... | *Ogmorhinus weddelli* |
| *D. coatsi* ... ... | (Rennie and Reid, 1912) | ... | Median | ... | 90, scattered | Absent, contain few eggs | ... | 60 | *Ogmorhinus weddelli* |
| *D. coniceps* ... ... | (Linstow, 1905) | Anterior | Median | Extends half-way between surfaces | ... | ... | Present | ... | *Phoca barbata* |
| *D. cordatum* ... | (Leuckart, 1863) | ... | Median | ... | 240 to 300; 2 separate lateral bands | 6 to 8, extending laterally to genital pore | ... | 70 to 80 by 50 | *Canis familiaris, Homo sapiens, Phoca barbata, P. greenlandica Trichechus rosmarus* |
| *D. cordiceps*... ... | (Leidy, 1872) | ... | ... | ... | ... | ... | ... | 70 by 35 | *Pelecanus erythrorhynchus* |
| *D. decipiens* ... | (Diesing, 1850) | ... | ... | Extends half-way between surfaces | ... | ... | ... | 50 by 60 | *Canis familiaris, C. lupus, Felis cattus, F. concolor, F. domestica, F. macroura, F. mellivora, F. mitis, F. onca, F. pardus, F. tigrina, mellivora ratel* |
| *D. dendriticum* ... | (Nitzsch, 1824) | ... | ... | ... | 470, 2 lateral fields joined anteriorly | 8 to 9 | ... | ... | *Larus argentatus, L. canis, L. ridibundus, Rissa tridactyla* |
| *D. ditremum* ... | (Creplin, 1825) | ... | ... | ... | 380 to 390, 2 lateral fields joined posteriorly and anteriorly | 7 | ... | ... | *Larus argentatus, Mergus merganser, M. serrator, Urinator arcticus, V. stellutus* |

| Species | | Genital pore | Uterine pore | Cirrus-sac | Testes | Uterine coils | Enlargement of uterus | Eggs (in $\mu$) | Host |
|---|---|---|---|---|---|---|---|---|---|
| *D. elegans* ... ... | (Krabbe, 1865) | ... | ... | ... | ... | ... | ... | 44 by 35 | *Cystophora cristata*, *Eumetopias jubata*, *Phoca vitulina* |
| *D. exile* ... ... | (Linton, 1892) | ... | ... | ... | ... | ... | ... | ... | *Larus californicus* |
| *D. fissiceps* ... ... | (Creplin, 1829) | ... | ... | ... | ... | ... | ... | ... | *Sterna hirundo* |
| *D. folium* ... ... | (Diesing, 1850) | ... | ... | ... | ... | ... | ... | ... | *Herpestes albicaudus* |
| *D. fuscum* ... ... | (Krabbe, 1865) | ... | ... | ... | ... | 5 to 7 (Railliet 10 to 12), lateral to male genital pore | ... | 55 to 60, non-operculated | *Canis familiaris* |
| *D. bians* ... ... | (Diesing, 1850) | Cirrus posterior to vagina (Ariola) | ... | ... | ... | ... | ... | 59 by 38 | *Monachus albiventer*, *Phoca barbata*, *P. hispida*, *P. vitulina* |
| *D. lanceolatum* ... | (Krabbe, 1865) | Anterior quarter | ... | ... | 180 to 312, 2 separate fields | 5 to 7, lateral to male genital pore | ... | 55 to 60 (Zschokke and Heitz 62 by 40) | *Phoca barbata* |
| *D. lasbleyi* ... ... | (Leiper and Atkinson, 1914) | Anterior | Lateral | ... | 2 separate lateral bands with 2 posterior ones | Simple | ... | 60 | *Ogmorhinus weddelli* |
| *D. latum* ... ... | (L.1758) | Anterior quarter | Median | Extends half-way between surfaces | 2 separate lateral bands | Numerous, extending laterally to genital pore | Absent | 67 to 70 by 48 to 54 | *Canis azarae*, *C. familiaris*, *Cystophora cristata*, *Felis concolor*, *F. domestica*, *F. hernandesii*, *F. macroura*, *F. mellivora*, *F. mitis*, *F. pardus*, *F. tigrina*, *Herpestes leucurus*, *Homo sapiens*, *Leptonyx monachus*, *Odobaenus rosmarus*, *Phoca barbata*, *P. hispida*, *P. vitulina*, *Phocaena phocaena*, *Ursus maritimus*, *Vulpes lagopus*, *V. vulpes* |

| Species | | Genital pore | Uterine pore | Cirrus-sac | Testes | Uterine coils | Enlargement of uterus | Eggs (in $\mu$) | Host |
|---|---|---|---|---|---|---|---|---|---|
| *D. macrophallus* ... | (Linstow, 1905) | ... | ... | ... | ... | ... | ... | ... | *Otaria ursina*, *Phoca barbata* |
| *D. mansoni* ... ... | (Cobbold, 1882) | ... | ... | ... | ... | ... | ... | 63 to 76 by 31 to 43 | *Canis familiaris* |
| *D. marginatus* ... | (Krefft, 1871) | ... | ... | ... | ... | ... | ... | ... | *Halmaturus*, sp. |
| *D. mobilis* ... ... | (Rennie and Reid, 1912) | Anterior | Lateral | ... | 2 bands joining anteriorly | 3 to 6 | ... | Fuhrmann 56 to 60 by 44 (Rennie and Reid 51 by 34) | *Ogmorhinus weddelli* |
| *D. nasuta* ... ... | (Rudolphi, 1802) | ... | ... | ... | ... | ... | ... | ... | *Parus major* |
| *D. parvum* ... ... | (Stephens, 1908) | Like *D. latum* | ... | ... | ... | ... | ... | ... | *Homo sapiens* |
| *D. perfoliatum* ... | (Railliet and Henry, 1912) | ... | Lateral | Extends half-way between surfaces | ... | Only one coil with eggs | ... | Fuhrmann 60 to 64 by 45 to 48 (Railliet and Henry 56 to 64 by 43 to 45) | *Ogmorhinus weddelli* |
| *D. polycalceollum* ... | (Ariola, 1896) | Anterior sixth | ... | ... | ... | 8 to 9, not lateral to genital pore | ... | 48 by 32 | *Phoca vitulina* |
| *D. podicipedis* ... | (Diesing, 1854) | ... | ... | ... | ... | ... | ... | ... | *Podiceps minor*, *P. rubricollis* |
| *D. pygoscelis* ... | (Rennie and Reid, 1912) | Very anterior | ... | ... | Scattered over proglottis | 6 to 7 | ... | 64 to 80 by 50 to 52 | *Pygoscelis*, sp. |
| *D. quadratum* ... | (Linstow, 1891) | Anterior quarter | Median | Extends half-way between surfaces | ... | 6 to 7 | Present | 54 by 44 (Fuhrmann 50 by 43) | *Ogmorhinus leptonyx* |
| *D. raillieti* ... ... | (Rátz, 1913) | ... | Median | Small | 300 to 500, 2 separate fields | 6 to 7 | Present | 62 to 70 by 37 to 54 | *Canis familiaris* |
| *D. resimum* ... | Railliet and Henry 1912 | ... | ... | ... | ... | ... | ... | ... | *Ogmorhinus leptonyx* |
| *D. römeri* ... ... | (Zschokke, 1903) | Anterior fifth | Median | To aporal surface | 600 to 1000 | 6 to 8, lateral to genital pore | ... | 62 by 39 | *Trichechus rosmarus* |

| Species | | Genital pore | Uterine pore | Cirrus-sac | Testes | Uterine coils | Enlargement of uterus | Eggs (in $\mu$) | Host |
|---|---|---|---|---|---|---|---|---|---|
| *D. rufum* ... ... | (Leiper and Atkinson, 1914) | ... | ... | ... | ... | ... | ... | 25 | *Ogmorhinus weddelli* |
| *D. schistochilos* ... | (Germanos, 1895) | ... | ... | ... | ... | ... | ... | 50 to 70 by 20 to 30 | *Phoca barbata* |
| *D. scotti* ... ... | (Shipley, 1907) | ... | Lateral | ... | ... | 4 | Present | Shipley 40 by 30, Fuhrmann 64 by 40 | *Ommatophoca rossi* |
| *D. scoticum* ... | (Rennie and Reid, 1912) | ... | Lateral | ... | ... | 5 to 6 | ... | 70 to 80 by 44 to 48 | *Ogmorhinus leptonyx* |
| *D. semiligula* ... | (Nitzsch, 1824) | ... | ... | ... | ... | ... | ... | ... | *Podiceps rubricollis* |
| *D. serratum* ... | (Diesing, 1850) | ... | ... | ... | ... | ... | ... | 64 by 46 | *Canis azarae*, *C. familiaris* |
| *D. similis* ... ... | (Krabbe, 1865) | ... | ... | ... | ... | ... | ... | 60 | *Vulpes lagopus* |
| *D. spiraliceps* ... | (Volz, 1900) | Uterine and genital pores one either side | ... | ... | ... | ... | ... | 36 by 32 | *Falco concolor* |
| *D. stemmacephalum* | Cobbold, 1858 | ... | ... | ... | ... | ... | ... | ... | *Phocaena phocaena* |
| *D. sulcatum* ... | (Molin, 1858) | ... | ... | ... | ... | ... | ... | ... | *Felis pardus* |
| *D. tectum* ... ... | (Linstow, 1892) | ... | Lateral or median | To medullary parenchyma | ... | ... | ... | 65 by 47 (Fuhrmann 65 by 44) | *Macrorhinus leoninus* |
| *D. wilsoni* ... ... | (Shipley, 1907) | ... | Lateral | Half-way between surfaces | ... | ... | ... | (Fuhrmann 48 to 52), Rennie and Reid 52 to 60 by 32 to 36 | *Ogmorhinus weddelli*, *Ommatophoca rossi* |
| *D. reptans* ... ... | (Diesing, 1854) | Anterior quarter | Median or lateral | Half-way between surfaces | 144 to 220, 2 separate bands | 4, not lateral to genital pore | Present | 53 to 59 by 36 to 40 | *Canis familiaris* |

## EXPLANATION OF PLATE IX

FIG. 1. *D. reptans:* scolex.

FIG. 2. *D. reptans:* mature proglottis. *c.s.*, cirrus-sac; *m.*, male pore; *ov.*, ovary; *t.*, testes; *u.*, uterus; *v.*, vagina; *v.*[1], vaginal pore; *vit.*, vitellaria.

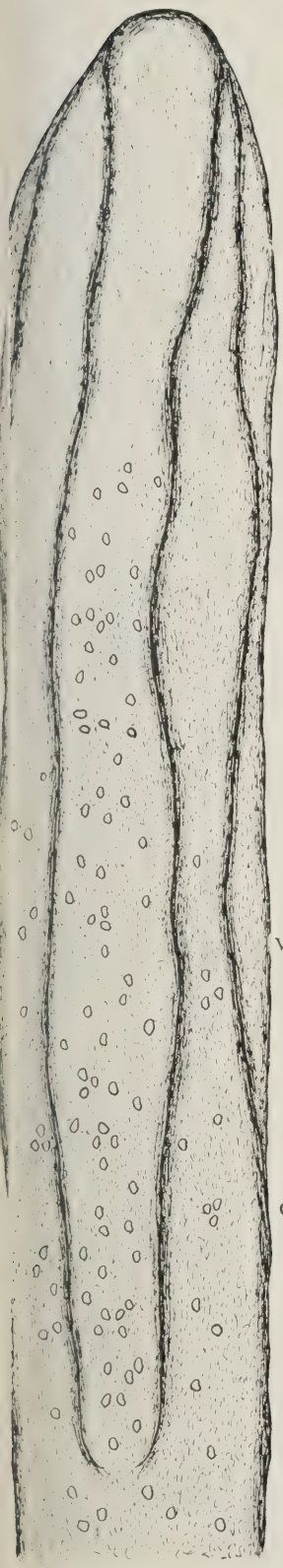

FIG. 1

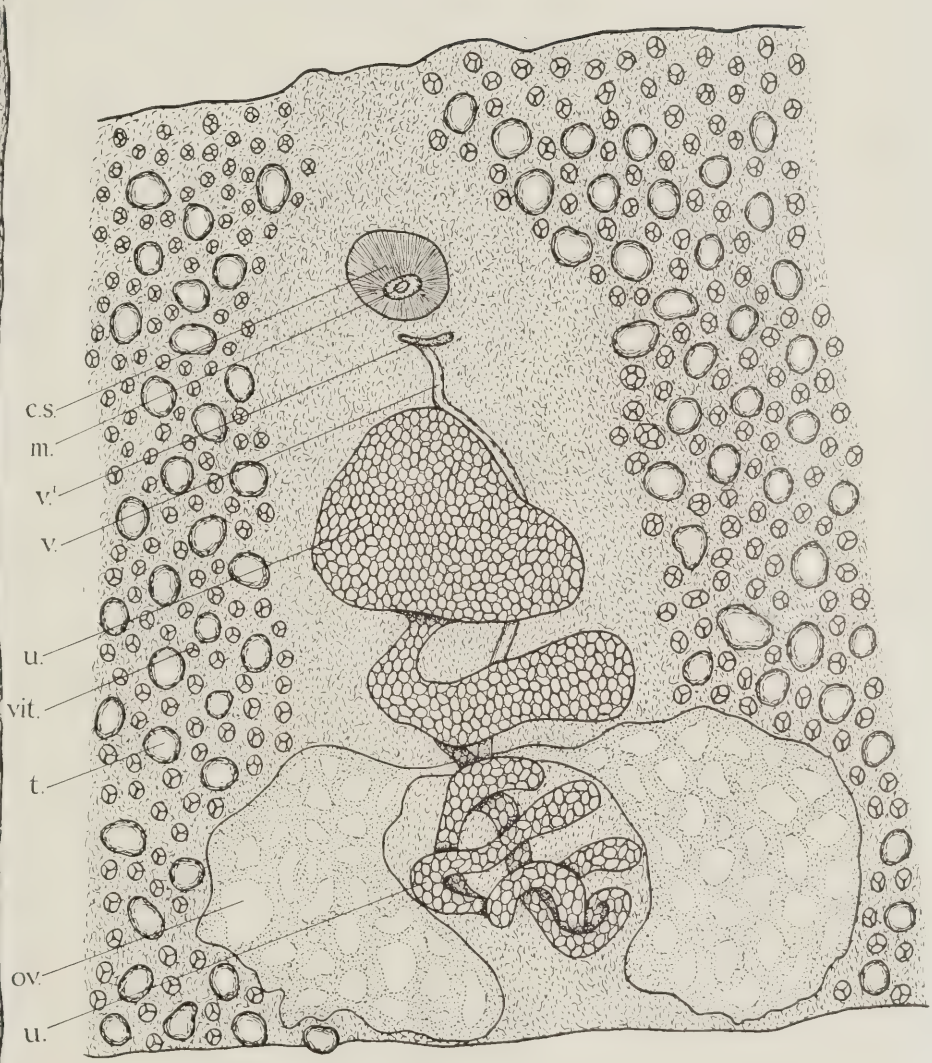


FIG. 2

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# A NEW VARIETY OF *GLOSSINA SCHWETZI*, NEWSTEAD & EVANS, FROM THE BELGIAN CONGO

BY

W. H. POTTS

(*Received for publication, 22 May, 1924*)

*Glossina schwetzi* var. *disjuncta* var. nov.

*Colour and pattern as in typical* G. SCHWETZI, *Newstead and Evans, but the distal processes of the harpes short and detached, and the median process more prominent.*

MALE. Length, 11·6 mm. ; length of palpi, 3·2 mm. ; width of head, 3·25 mm. ; length of wing, 10·6 mm.

*Genital Armature* (fig. 1, *A*). Differing from that of typical *G. schwetzi* in the following characters :—

(1) The *harpes* bears three pairs of processes (*h*.1 ; *h*.2 ; and *h*.3), the distal or third pair of which (*h*.3) is detached from the main body of the harpes, and, instead of being the longest, is barely as long as the second or middle pair (*h*.2).

(2) The *median process* (*m.p.*) is larger, and projects well beyond the inferior claspers (*i.c.*), between which it is situated.

An illustration of the harpes of *Glossina schwetzi* (fig. 1, *B*) has been added in order to facilitate the comparison of the two armatures.

The above description is based on one specimen, a male, in a collection sent to this School for identification by Dr. Schouteden from the Museum of the Belgian Congo, Tervueren. The fly is one of two accompanied by the following data :—' Sur buffle tué à Buku Kaie, 26.6.21, Rodhain.' The other fly proved to be a typical male *G. tabaniformis*.

The Type specimen is being sent back to the Museum of the Belgian Congo, Tervueren.

It is with great pleasure that I take this opportunity of thanking Professor Newstead, for his kind advice and assistance.

Fig. 1, *A*. Male Genital Armature of *Glossina schwetzi* var. *disjuncta* var. nov. (× about 60). *B*. Portion of male genital armature of *Glossina schwetzi*, Newstead and Evans (× about 50). *h*.—harpes; *h*.1, *h*.2, *h*.3—first, second, and third pairs of processes of the harpes; *i.c*.—inferior claspers; *m.p*.—median process; *m.p.l*.—inferior median process; *s.c*.—superior claspers; *v*.—vesica.

# THE CRESCENT AND THE RED CELL

BY

J. W. W. STEPHENS

AND

R. M. GORDON

(*Received for publication* 26 *May*, 1924)

PLATES X-XIII

The following observations were made on a series of thin blood films from a case of malaria in the Federated Malay States. The films were made from 6.8.23 to 14.8.23, during which period the patient was taking quinine grains 20 daily.

Lawson (1911-1920) in a series of papers on the morphology of the malaria parasite, has published a number of beautiful photographs of crescents showing the variable relationship existing between the crescent and the red cell ; nevertheless, authors continue to publish illustrations representing practically only one stereotyped form.

In the present paper accordingly we have thought it worth while to draw attention to this variability.

We had also hoped to throw light on the vexed question as to whether the malaria parasite, in so far as the crescent is concerned, is in or on the red cell.

We may state at once that we consider that the specimens show clearly that in many cases the poles of the crescent project beyond the red cell, but we have been unable to determine the relationship of the body of the crescent to the red cell, although the general impression left on our minds after examining a very large number of crescents during the last three months is that the body also is extracellular.

Although we are unable to explain how the ' loop-form ' which is probably the commonest form, and which is the one almost

invariably shown in the text books, arises, yet we can trace a transition from the stage in which the red cell stains uniformly, having no portion decolorised, to the loop-form in which the red cell is entirely decolorised except for a skeleton outline or rim.

Lawson has already figured most if not all of the forms we propose to describe, but we think that a diagrammatic representation gives a clearer idea of the appearances actually seen, than does a photograph however beautifully executed.

It will be seen that the majority of the crescents figured are females. This is due to the fact that we frequently experienced difficulty in distinguishing the line of demarcation between the male crescent and the red cell as both stained an almost equally intense red.

This intense red staining of crescent-infected erythrocytes is peculiar and further they do not show any appearance resembling the stippling (Stephens' and Christophers' dots) characteristic of the ring forms of *Plasmodium falciparum.*

With regard to the crescent itself, it appears to us to be a flat ribbon-shaped structure, the ends of which, in thick portions of a film are generally incurved, while in thin portions the crescent lies flat.

We did not observe any forms that we could interpret as young or developmental forms.

The illustrations were made diagrammatically at a magnification of about 4,000 diameters. They may be arranged in four groups.

PLATE X (figs. 1-12)

*Group A. Those in which the red cell stains uniformly, presenting a solid appearance.*

Figs. 1, 2, 8, 9. The surface of the crescents appears to show buds or projections.

Fig. 6. Shows a projection at one pole. From its staining character it appeared to be red cell, but in many cases it was impossible to be certain as to the nature of these 'buds.'

Figs. 7-11. The poles of the crescent project beyond the red cell.

Fig. 12. Shows folding of the crescent.

## Plate XI (figs. 13-24)

*Group B. Those in which the red cell is partly decolorised.*

Figs. 13-18. More or less of the red cell is decolorised.

Figs. 19-24. A gradual transformation to the loop-form.

Fig. 23. Shows a solid wedge of non-decolorised red cell extending across the decolorised area.

Fig. 24. A somewhat similar appearance.

## Plate XII (figs. 25-36)

*Group C. Those in which the greater part of the red cell is decolorised and in which part of the circumference of the red cell takes the form of a loop usually corrugated and generally subtending the concavity of the crescent.*

Fig. 25. A typical loop-form. The crescent is completely surrounded by a rim of red cell, and the loop arises from two triangular thickenings on one side of it. These thickenings are situated not at the poles of the crescent but at some little distance therefrom. The rim of red cell presents coarse corrugations usually most prominent on its convexity, while the loop presents a series of finer corrugations. The loop bounds an area almost decolorised, but a granular basis can be frequently recognised.

Fig. 26. The crescent has pointed ends and projects beyond the red cell margin.

Fig. 27. A double-loop, a not uncommon form. The poles of the crescent are free.

Fig. 28. A similar form. One pole projects.

Figs. 29, 30. Two loops, both on the same side of the crescent. A possible interpretation is that the crescent lies between the folded red cell.

Figs. 31-35. Irregular loop formation.

Fig. 32. The outline of the red cell can be seen crossing the body of the crescent.

Fig. 36. Unusual position of the loop.

## Plate XIII (figs. 37-48)

*Group D. Miscellaneous forms.*

Figs. 37-45. The crescents are folded.

Figs. 37-39. The red cell is not decolorised.

Figs. 40-45. Various degrees of folding of the crescents and decolorisation of the red cell.

Fig. 46. A crescent with pointed poles (cp. fig. 26). These forms were encountered not infrequently. Their significance is unknown.

Figs. 47, 48. Double infection of the red cell. In both cases a male and a female crescent occurred in each red cell.

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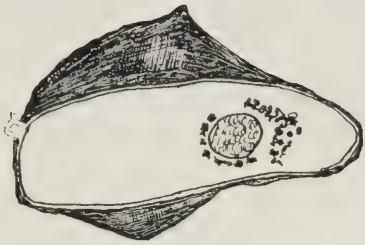

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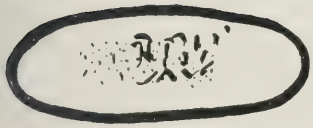

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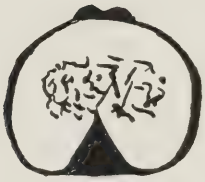

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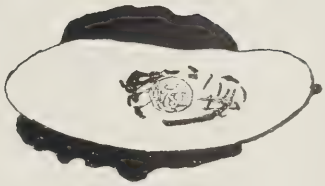

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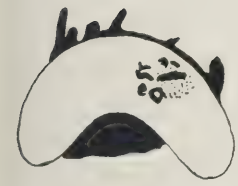

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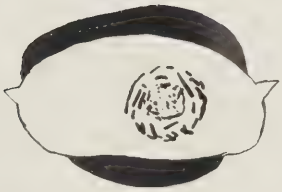

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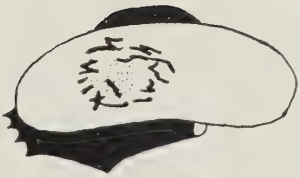

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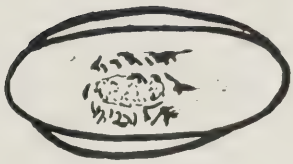

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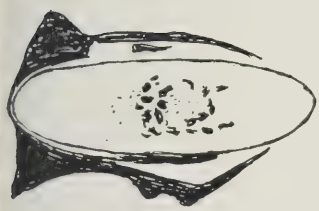

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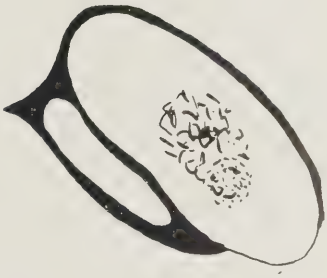

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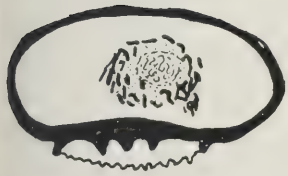

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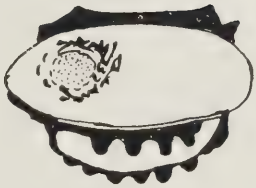

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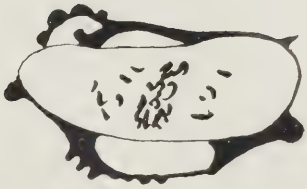

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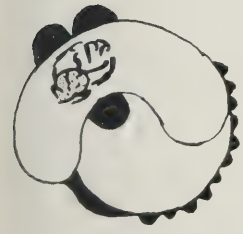

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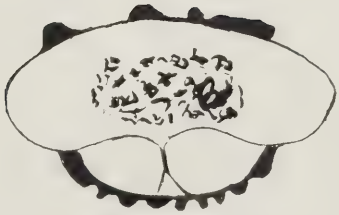

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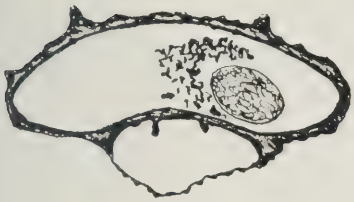

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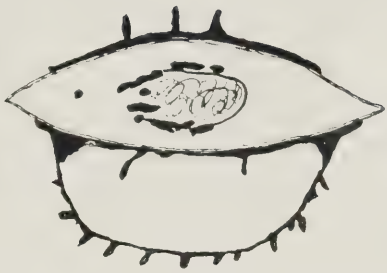

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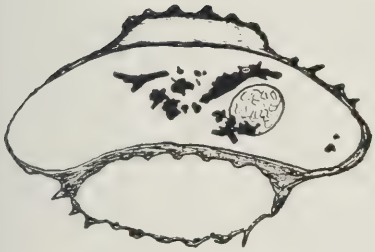

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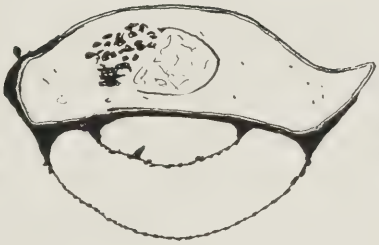

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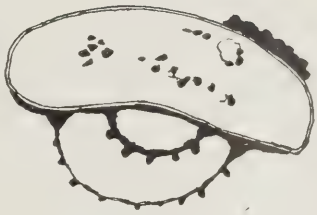

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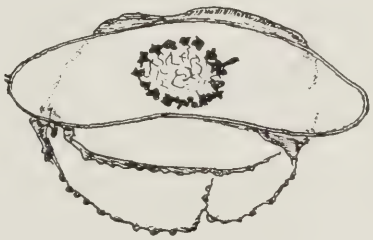

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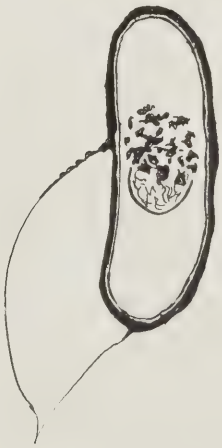

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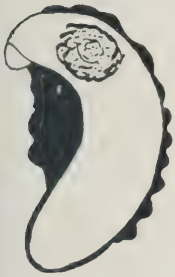

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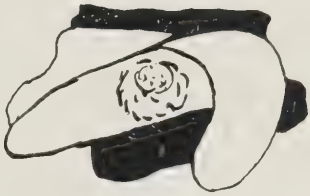

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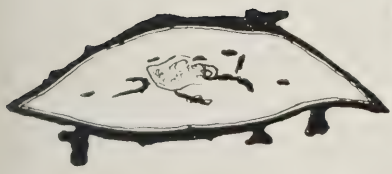

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# HUMAN SCHISTOSOMIASIS DUE TO *S. HAEMATOBIUM* IN SIERRA LEONE

BY

D. B. BLACKLOCK, M.D.

AND

M. G. THOMPSON, M.B., B.Sc.

(*From The Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication* 30 *May*, 1924)

PLATE XIV

During December, 1923, and January and February, 1924, an investigation was carried out into the prevalence of human Schistosomiasis in certain districts of the Protectorate of Sierra Leone. A Report on the subject has been submitted to the Government of Sierra Leone and will be published in the *Annual Medical and Sanitary Report* of the Colony.

The Report dealt with :

1. The prevalence of Urinary Schistosomiasis in the various districts traversed, and among the various tribes examined.
2. The discovery of the intermediate snail host ; this was proved to be *Physopsis* c.f. *globosa*, Morelet.
3. The rarity of snails of the genus *Planorbis* and the absence of Intestinal Schistosomiasis.
4. Tribal customs which facilitate the spread of infection.
5. Sanitary condition of the villages.
6. Recommendations for the control of the disease.

In the present paper, it is proposed to deal with certain morphological observations on the cercariae found in the intermediate host, and with some facts relating to the bionomics of this snail which were not dealt with in the Government Report.

Major Connolly, to whom the various snails collected were sent and who kindly identified them, proposes to publish a complete account of all the snails found during the expedition and to include in it all previously known forms found in Sierra Leone.

## DESCRIPTION OF THE CERCARIA OF *S. HAEMATOBIUM*

*Examination.* The snails were placed each in a separate small test-tube in clean water ; the cercariae which emerged were examined with a hand-lens of 20 magnification in order to study their movements. The fluid containing them was then pipetted off as required and mounted as a cover slip preparation, and the details of structure noted. It was found that cercariae which emerged just after clean water was added to the snails were more easily studied than those which emerged into water which had stood some time ; in the latter case the medium was not so translucent as in the former. The living preparation gave the most satisfactory results for the study of the suckers, the secretory glands and the excretory system, especially the flame cells. On the other hand, fixation with subsequent staining gave better results in studying certain features of the secretory gland cells and their ducts, the nervous system, the genital cells and cuticular spinulation. Specimens stained *intra vitam* were examined, the stain chiefly used being very dilute Leishman's stain.

*Movement.* In a tube of water the cercariae swim actively but intermittently ; as a rule, active progression is towards the surface of the water, the tail end leading. On cessation of swimming the furcal rami are usually held at right angles to the tail stem, and the animal begins to sink slowly. It was observed that if the rami were maintained at right angles (fig. 3, VI) the animal would quickly resume its upward movement towards the surface after a very short rest. If, on the other hand, the animal allowed the rami to curl inwards towards the tail stem in the shape shown in fig. 3, VII, it would fall rapidly in the water for a long distance. The downward movement was accelerated by curling the rami more tightly, until they appeared as two small knobs at the end of the tail stem.

Under a coverslip in a drop of water, the movements depend upon the amount of fluid ; if the fluid is deep the animal moves with great rapidity and passes out of the field. If the fluid is drained away slowly with blotting-paper, the animal attaches itself to the glass and progresses slowly by alternate protrusion of the cephalic end and drawing up after it of the body ; the tail, not being compressed by the slip, moves very actively at intervals ; it separates very easily

from the body in such conditions and was observed to retain the power of independent movement for a considerable period, up to ten minutes after separation. This independent movement was most frequently slow, affecting chiefly the base and rami, but sometimes a very active lashing movement affecting the whole tail was observed; in no case was translatory movement of the separated tail seen. The separated body, however, is capable of active progression and in this the ventral sucker is strongly protruded and adheres to the glass surface. Occasionally the animal appears to have difficulty in releasing its hold by the ventral sucker and the anterior end of the body is turned so as to release the ventral sucker by the help of the anterior protrusible organ. During the various movements, the body alters its shape constantly and remarkably and the anterior protrusible organ is pushed vigorously against any obstacle; the margins of the ventral sucker appear circular, oval, or linear in different views.

The appearance of the moving body under a coverslip changes so rapidly and the shape of the important organs alters so completely from one moment to another, that it frequently has little resemblance to the diagram which is reconstructed to represent the animal; the attached tail undergoes equally great variations in appearance, even when moving fairly slowly. It is only when the animal's movements are slowed down by removal of fluid from under the coverslip that the more important organs can be studied, namely, the anterior and ventral suckers and particularly the secretory glands.

*Measurements.* It is extremely doubtful if the measurements of the length or width of the body and the tail and rami of such cercariae can have any other than a crude comparative value. This statement is based on the fact that the animal in the living state is capable of such remarkable variations in the size of its body, tail-stem and furcal rami. The states of contraction and extension which are so obvious in the living animal are represented in fixed preparations. While a general contraction of the animal may be the result of applying to it certain fixatives, the degree to which the contraction affects different parts of the body will depend on the state of those parts of the body at the moment of application of the fixative. This is illustrated in Table I, in which are given the maximum and minimum sizes of parts of the cercariae.

TABLE I

The maximum and minimum sizes (in $\mu$) of different parts of the cercaria in specimens, living, or fixed in different ways.

| Measurement | Part of cercaria | Living | | Fixed in Schaudinn's fluid | | Fixed in 5 % formalin | |
|---|---|---|---|---|---|---|---|
| | | maximum | minimum | maximum | minimum | maximum | minimum |
| Length ... | Body ... | 242 | 105 | 196 | 121 | 189 | 114 |
| | Tail stem ... | 253 | 186 | 232 | 147 | 228 | 136 |
| | Furcal rami | 92 | 80 | 77 | 44 | 94 | 52 |
| Width ... | Body ... | 92 | 35 | 80 | 58 | 70 | 40 |
| | Tail ... | 46 | 23 | 42 | 33 | 47 | 24 |

It is evident that a comparison of measurements which show such variation in the extremes under each method can have only limited value. Even if we take averages of one method of fixation, *e.g.*, 5 % formalin, it is of little advantage, because the averages may be deceptively alike for any two groups, while the individuals vary enormously. Thus, taking two sets of fifteen examples fixed by this reagent from among those for which the extremes are given above, the result shown in Table II was obtained.

TABLE II.

Showing the average measurements (in $\mu$) obtained from formalin fixed specimens of *S. haematobium*.

| | Average length in $\mu$ | |
|---|---|---|
| | 1st set of 15 | 2nd set of 15 |
| Body ... ... ... ... ... ... ... ... | 144 | 144 |
| Tail stem ... ... ... ... ... ... ... ... | 184 | 177 |
| Furcal rami ... ... ... ... ... ... ... | 63 | 67 |
| | **Average width in $\mu$** | |
| | 1st set of 15 | 2nd set of 15 |
| Body ... ... ... ... ... ... ... ... | 57 | 59 |
| Tail ... ... ... ... ... ... ... ... | 32 | 34 |

The statements of Soparkar (1921) with reference to the cercaria of *S. spindalis* that

'the fully extended body often measures twice the contracted,'

of Sewell (1922) with reference to *Cercaria indica* XXX that it measures in body length extended 210$\mu$ and contracted 90$\mu$, bear out the statement of Cort (1919) with regard to *S. japonicum* cercariae, that

'measurements of the cercaria of *S. japonicum* are very unsatisfactory data for comparison.'

Measurements of *S. mansoni* cercariae given by Iturbe and González differ so considerably from those given by Faust (1919) as to support the contention that measurements are of little service in differentiating the cercariae mentioned. The difficulty of attaining any standard agreement in regard to the measurement of such organs as the suckers is as great as that attending the measurement of the body, tail stem and of the furcal rami.

*Cuticle.* On the body, tail and on the furcal rami are present small backwardly directed spines; these are present also on the ventral sucker, but were not seen on the oral sucker. The spines are longer and more sparsely distributed on the base of the tail stem; subcuticular muscles both longitudinal and circular are present and are strongly developed in the region of the ventral sucker. There are no eyespots.

*Anterior sucker.* This is of elongated oval shape with a definite, thick, muscular wall, and is capable of a considerable range of movement, chiefly extension and contraction. In the substance of the sucker there is a homogeneous looking mass of a semi-fluid consistency, in the posterior part of which there are numerous small oval cells. There is no evidence of the presence of a nucleus, or duct, or secretion which would entitle this body to be termed a gland analogous to the head gland described by the Japanese observers in the case of *S. japonicum* cercariae. The mouth aperture is subterminal and is usually seen as a small circular opening from which a tube leads backwards into the body. At the anterior margin of the sucker, considerably anterior to the mouth opening, are situated the openings of the secretory gland ducts. Each duct opens at the tip of a minute refractile spine out of which the secretion can easily be seen passing in living specimens. From this point the

ducts can be traced backwards through the substance of the oral sucker, on its ventral aspect (fig. 2). There are five ducts on each side of the middle line. The ducts are markedly constricted where they pass through the wall of the oral sucker.

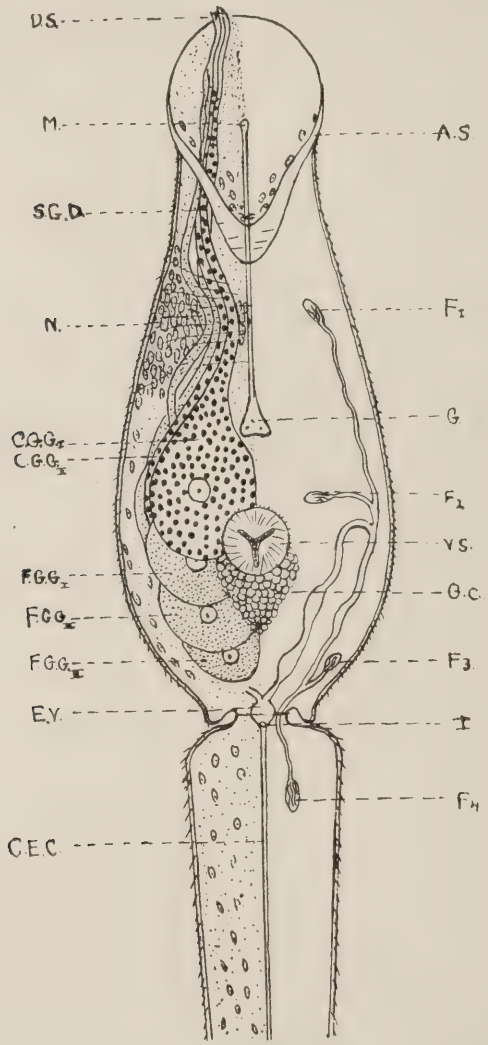


Fig. 1. Diagram of the Cercaria of *S. haematobium*. *D.S.*—Duct spines; *M.*—Mouth; *S.G.D.*—Secretory gland duct; *N.*—Nervous system; *C.G.G.*—Coarsely granular gland; *F.G.G.*—Finely granular gland; *E.V.*—Excretory vesicle; *C.E.C.*—Caudal excretory canal; *F.*—Flame cell; *A.S.*—Anterior sucker; *V.S.*—Ventral sucker.; *G.*—Gut; *G.C.*—Genital cells; *I.*—Island of excretory vesicle.

*The Secretory Glands.* The posterior two-thirds of the body are almost entirely occupied by pairs of unicellular glands. These glands in cercariae have received many names, *e.g.*, mucin, mucoid, poison, salivary, venom, lateral, proteolytic, cephalic, and secretory. Cort (1919) prefers to use the term 'cephalic,' since their ducts open at the anterior tip of the cercaria. As the term 'head gland' is used by him for the gland in the anterior sucker of the cercaria of *S. japonicum*, this nomenclature is confusing. We use the term

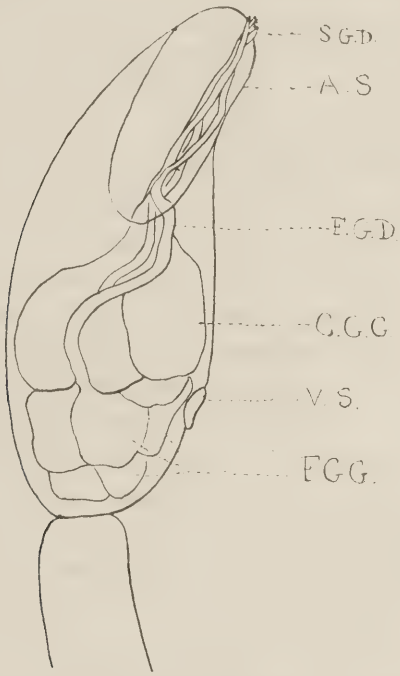


FIG. 2. Lateral view of cercaria of *S. haematobium*, drawn with the camera lucida, showing the course of the secretory gland ducts.

secretory glands for these body glands, in *S. japonicum*. The secretory glands number five on each side; they are unicellular, each with a single nucleus and definite nucleolus. The protoplasm of the cells contains granules; these are of large size in the anterior two pairs of gland cells, and small in the posterior three pairs of gland cells. The two pairs of anterior cells are large, generally flask-shaped, and lie about the same level, that is, just anterior to the ventral sucker. The three pairs of finely granular cells lie

posterior to them ; they are of smaller size and overlap each other somewhat.

The ducts from the secretory glands pass forwards ; widely separated at their origin from the individual cells, they become approximated in the region of the nerve cells and separate again before entering the anterior sucker. The ducts from the coarsely granular cells are also coarsely granular throughout their extent in the body and for a short distance in their course in the oral sucker ; they lie more ventrally and closer to the middle line than do the other ducts. The division of these glands into coarsely granular and finely granular is a constant and easily recognised fact in the living specimen.

The affinities of these cells for stains are variable, Stained with eosin for a short time, the anterior glands and their ducts often take on the stain diffusely, while the posterior finely granular cells remain unstained. In formalin specimens stained for prolonged periods with eosin, not only the coarsely granular cells, but the finely granular cells and their nuclei are well stained. In specimens stained with Ehrlich's haematoxylin, the anterior coarsely granular cells remain as a rule unstained and appear as a clear area in front of the ventral sucker, whereas the posterior finely granular cells with their ducts are deeply stained. With iron alum haematoxylin, the nuclei of the secretory gland cells both anterior and posterior are well defined and can be easily counted on careful focussing. The results obtained by staining were, however, not constant, even in fully developed cercariae. It is possible that the staining affinities of these glands vary to some extent with variation in their state of secretory activity. Faust and Meleney (1924) state that in the case of *S. japonicum* cercariae—in which the cephalic glands are all of one type—these glands are basophilic in reaction in very young cercariae. On reaching maturity the reaction changes from basophilic to oxyphilic, which condition prevails in the mature cercaria. Cort (1919) describing the same glands also in the cercaria of *S. japonicum*, states that they are stained a light blue with haematoxylin. It is interesting to note that the head gland of *S. japonicum* cercariae is described by Cort (1919) as taking ' the red stain erythrosin,' while Faust and Meleney (1924) state that it is slightly basophilic. Enough has been said to show that the acidophilic or

basophilic character of these cells is not a sufficiently definite nor stable one to justify its use as a distinguishing specific feature.

*Alimentary Canal.* This is simple in type and consists of a straight, narrow tube which passes from the mouth posteriorly and dorsally and ends in a small heart-shaped dilatation ; there is no pharyngeal bulb.

*Excretory System.* This commences in four flame cells on each side ; three of these are situated in the body as follows :—The anterior one half-way between the anterior extremity and the ventral sucker, the middle one on a level with the anterior margin of the ventral sucker, the posterior one about the level of the most posterior of the secretory glands. The fourth flame cell is situated in the tail near the base (fig. 1). From each flame cell a capillary leads off ; those from the anterior and middle body flame cells join ; those from the tail and posterior body flame cells also join. The two collecting tubes so formed unite at the level of the ventral sucker, curve ventrally and then pass towards the junction of the tail and body. Here they enter a globular excretory vesicle. From this a tube arises posteriorly which runs down the centre of the tail stem ; it divides into two branches just above the tail fork and each branch passes down one furcal ramus to open at its tip in a definite papilla. The flame cells in the body are seen most clearly from the dorsal aspect.

The excretory system corresponds closely with that of the cercaria of *S. japonicum* described by Cort and with that of *Cercaria indica* XXX described by Sewell. Cort (1919) found in addition ' two ciliated areas on each side near the ends of the sides of the bladder ' Sewell (1922) found two dilatations on each canal, each dilatation being provided with a ' flagellum.' The position of the flagellum in *cercaria indica* differs from that of the cilia in the cercaria of *S. japonicum*, as shown by the diagrams. We have not been able to satisfy ourselves of the presence of any flagellated or ciliated area in the collecting tubes of *S. haematobium*. In one specimen under observation from the lateral view, an appearance was noted which suggested the presence of such an area, but on rolling the specimen slightly it was found that the flickering movement seen was due to the movements of the flame cells of the opposite side showing through the body protoplasm.

*Genital System.* A triangular shaped mass of small cells just posterior to the ventral sucker represents the genital system.

*Nervous System.* There is in the region behind the anterior sucker a mass of cells which is wide at the lateral margins and tapers rapidly to cross the middle line as a narrow band ; in the substance of the mass, faint transverse lines are seen ; this appears, from analogy with other cercariae, to be the precursor of the nervous system of the adult.

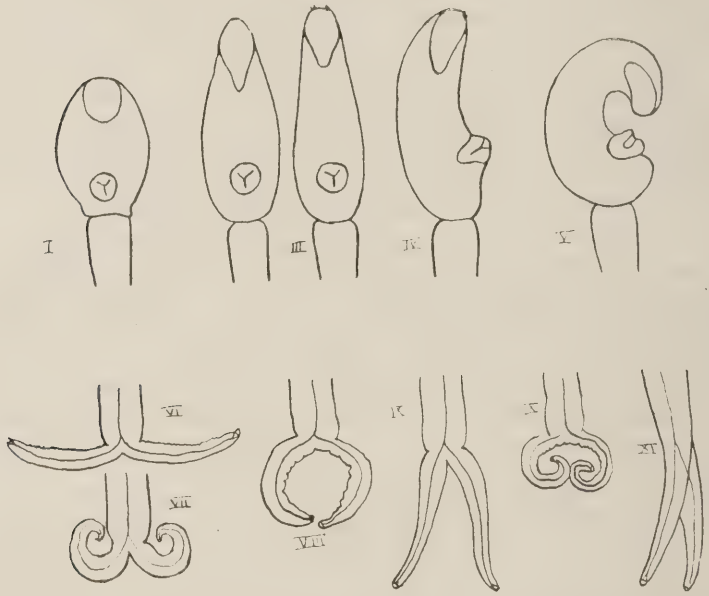


Fig. 3. Diagrams illustrating various positions assumed by the cercariae of *S. haematobium.*

*The Ventral Sucker.* This is small, being less than half the diameter of the anterior sucker. It is situated on the posterior fourth of the body. Its most characteristic feature is the remarkable degree to which it can be protruded. Seen from the side it has a cup-shaped appearance while from the ventral aspect it appears as a muscular ring with radiating lines and a central Y-shaped aperture. It is used by the animal for attaching itself to objects ; the anterior sucker is used freely as a penetrating organ.

## THE DIFFERENTIAL CHARACTERS OF THE CERCARIAE OF THE THREE HUMAN SCHISTOSOMES

It has been shown that in the Schistosome cercaria from Sierra Leone here described, there are two sets of secretory glands comprising two with coarse granules and three with fine granules on each side of the body. The number, morphology, and position of the secretory glands in this cercaria are such as to distinguish it definitely from the cercaria which Faust describes as that of *S. haematobium*. On the other hand it agrees somewhat more closely, in the secretory gland arrangement, with his description of *S. mansoni*. He describes, however, six pairs of 'mucin glands' for the *S. mansoni* cercaria. In the number of glands the Sierra Leone cercaria agrees with the number for *S. japonicum* given by Cort; it differs in having the glands divided into coarsely and finely granular cells.

Faust (1919) gives the following table :—

TABLE III.

Diagnosis of Species of Human Schistosome Cercariae.

| | *S. haematobium* | *S. mansoni* | *S. japonicum* |
|---|---|---|---|
| Size (in $\mu$) : | | | |
| Body ... ... | 240 × 100 | 140 × 60 | 100 to 210 × 66 |
| Tail trunk ... | 200 × 47 | 200 × 27 | 150 × 20 |
| Furci ... ... | 80 to 100 long | 50 long | 75 long |
| Oral sucker ... | 60 in transection × 64 in length | 30 to 34 in transection × 30 to 34 in length | 33 in transection × 54 in length |
| Mucin glands ... | 3 pairs with large nuclei and granular acidophilic cytoplasm. | 2 pairs with large nuclei and granular acidophilic cytoplasm ; 4 pairs with small nuclei and basophilic slime contents. | 5 pairs with large nuclei and granular acidophilic cytoplasm. |
| Mucin ducts ... | Moderately thick. | Very thick. | Very thick. |
| Duct openings ... | At anterior end of oral sucker ; capped by 3 pairs of hollow piercing spines. | At anterior end of oral sucker ; capped by 6 pairs of hollow piercing spines. | At anterior end of oral sucker ; capped by 5 pairs of hollow piercing spines. |
| Germ cells ... ... | Several large cells posterior to acetabulum | Many cells at posterior end of body | Clustered mass of cells just behind acetabulum |
| Parthenita ... ... | Sporocyst | Sporocyst | Sporocyst |

Let us now consider these diagnostic points individually. Reasons have already been given for doubting the value of the *measurement* criterion.

The thickness of the *mucin ducts*, which are described by Faust as :—*haematobium*, moderately thick, *mansoni*, very thick, *japonicum*, very thick, can have no significance, because, as has been shown, the ducts of the secretory glands of the cercaria undergo great and definite variation in thickness at certain points during their course forwards to the cephalic extremity. The *duct openings* correspond in Faust's table to the number of glands allotted by him to each species ; they are each capped by a hollow piercing spine, and are situated at the anterior end of the oral sucker. The description of the position and number of the *Germ cells* given is not such as to afford much assistance in diagnosing the three species, nor is it of help in allocating the present cercaria. 'Several large cells posterior to acetabulum,' 'many cells at posterior end of body' and 'clustered mass of cells just behind acetabulum' may represent very similar numbers and arrangement of the Germ cells. The *Parthenita* in all cases is Sporocyst. It is seen therefore on analysis of this Table that the supposed differences which are set out so far are indeed trifling and are valueless for diagnosis. Only in one particular, namely, the number and character of the *mucin glands*, does there appear to be a point of some value—*haematobium* three pairs, *mansoni*, six pairs, and *japonicum* five pairs.

It is legitimate at this stage to examine the basis upon which this diagnostic table was compiled. The most complete description of the cercaria of *Schistosoma japonicum* then existing was that of Cort (1919). When Leiper (1915) differentiated by experimental means the two forms *S. haematobium* and *S. mansoni*, he did not give any such detailed account of the cercaria as has since been attempted, for he considered that animal experiment gave the only satisfactory differentiation.

Faust (1919) studied specimens of cercariae from Natal believed to be those of *S. haematobium* and gave a description which is too long for complete quotation.

'The oral sucker leads into a digestive tract without any evidence of a pharynx. An oesophagus runs backwards into *ceca which extend about three-fifths the distance caudad* (italics not in original). Paired groups of mucin glands empty their slimy contents at the outer margin of the oral sucker. Each duct opens thru' a hollow

piercing spine which caps the duct. Each group can be traced back to three mucin glands in the region of the acetabulum. These cells have loosely scattered granules in the cytoplasm and large nuclei. No other mucin glands have been found. Several germ cells have been found in the region of the body posterior to the acetabulum. The number is considerably in excess of the number of testes in the adult worm.'

This description, it will be observed, was made from dead material sent by Dr. Cawston; the material also contained cercariae which Faust described as those of *S. mansoni*.

There are certain facts of great relevance which may be brought into evidence with regard to the description of *S. mansoni* given by this author. He examined specimens of *S. mansoni* cercariae from Caracas and found that

'the mucin glands consist of only two pairs of cells of the granular type, but, in addition, four pairs of a non-granular type, somewhat smaller and surrounding the granular cells.'

This description is markedly at variance with that given by Iturbe who figures the glandular system of *S. mansoni* as having only three glands on each side. Faust (1920) states that

'this method of distinguishing between these species of larvae makes it possible to diagnose two species in material which Dr. F. G. Cawston has sent the writer from Natal, namely, cercariae of *Schistosoma haematobium* and those of *S. mansoni*. The latter species corresponds both by structural and micro-chemical tests to Iturbe's species from Venezuela.'

When we pursue this matter a little further, we find that Cawston (1920) is of the opinion that 'the statement must be taken with caution.' Later Cawston (1922) makes further reference to this subject and to Faust's diagnosis, as follows:

'In a specimen of *Physopsis africana* which I sent him from Natal, Faust has reported the presence of the cercariae of *S. haematobium* and *S. mansoni*, as well as *Cercaria octadena* which he regards as a developmental stage of *S. bovis*—it is difficult to understand how one individual snail from the Durban suburbs can have been exposed to infestation by the miracidia of all three Schistosomes.'

Faust (1921) met this criticism in so far as *S. mansoni* was concerned by pointing out again that the cercaria described by him for South Africa corresponds in all critical points with his description of the one from Venezuela which is known experimentally to be the larva of *S. mansoni*. Some explanation of this kind was clearly demanded as Cawston had drawn attention to the curious fact that although *S. mansoni* infection in human beings was not known to him to occur endemically in Natal, yet a single specimen of a snail harboured

the cercaria of *mansoni* as well as that of *haematobium* and of *bovis*. Further there was the additional cause of surprise to Cawston that the cercaria discovered in such interesting circumstances should be found in a type of snail host, *Physopsis africana*, which was never previously reported to contain the cercariae of *S. mansoni*. Faust would certainly appear to have been fortunate in obtaining in one snail specimen no less than three distinct species of schistosome cercariae and the fact that two of these were of great importance as being the early stages of Schistosomes affecting human beings gave his findings additional weight. The discovery by this means of the hitherto unsuspected endemic existence of *S. mansoni* in Natal was possibly of great epidemiological significance. Incidentally the revelation of a new type of snail intermediate host for *S. mansoni* was not less striking.

We find then that Faust's description of the cercaria of *S. haematobium*, is based on no definite evidence that he was in fact dealing with the cercaria of this species. It was not proved experimentally to be associated in any way with *S. haematobium*. The only evidence that it might probably be the cercaria of this adult worm was that it came from a likely snail from an endemic area. But the very fact that it came from a snail which was an intermediate host of *S. haematobium*, appeared to Cawston somewhat strong evidence against there also coming from this snail a cercaria which was the young stage of *S. mansoni*. In 1921 Faust, to meet the objections of Cawston, and in order to clinch the argument finally, made the following statement with regard to the same snail :—

> 'Furthermore, I have actually seen the lateral spined eggs of *S. mansoni* preserved in the liver gland of *Physopsis africana* which Dr. Cawston collected from Ottawa, Natal, and later sent me.'

Faust does not suggest any possible or probable explanation as to the means by which these eggs arrived in the position stated in the snail.

Thus detailed descriptions of cercariae which he presumed were those of *S. haematobium* and *S. mansoni* have been made by Faust ; in both cases from preserved material and without the advantage of studying living material. The great necessity of examining living specimens is emphasized by Cort, Sewell, Soparkar, and others who have added materially to our knowledge of the minute anatomy of cercariae.

## THE IDENTITY OF THIS CERCARIA FROM SIERRA LEONE

We are faced by the fact that the description of the cercaria as given above differs from the description of the cercaria of *S. haematobium* given by Faust and reproduced in various text-books. It differs from his description not only in the number and character of the secretory glands but also in the anatomy of the alimentary canal. If this author's description is correct, then we are dealing here not with *haematobium* but with some other species. But the evidence provided by the epidemiology, by the experimental infection of snails, by the type of snail infected, and last of all by the results of the experimental infection of laboratory animals appears too convincing to be set aside, and we have little doubt that this cercaria is, in reality, that of *S. haematobium*.

The evidence which can be given to prove that it is the cercaria of *S. haematobium* is :

*A.* Incidence in Human Host.

Among the population examined no schistosome ova other than those with terminal spines were found. In a total of 180 faeces examinations these ova were found once ; among a total of 808 urine examinations they were found in 305 persons.

*B.* Experimental Infection of *Physopsis* c.f. *Globosa*, Morelet

Nine specimens of this snail from an uninfected area were kept in separate tubes and examined daily. Each day fresh water which had been boiled and then allowed to cool was used to replace that in which the snails were kept. The snails did not give out any cercariae ; controls which were dissected did not show any infection. The terminal spined ova from the urine of an infected case were then added to the tubes in which the snails were kept, on two successive days. Active miracidia were seen attaching themselves to the snails. The water thereafter was daily examined as before and then changed when found free of cercariae. On the fifteenth day after the second exposure, cercariae were found in the water in which one of the snails was. Two others also were found to be infected during the next examination. Dissection of the remainder of the snails did not reveal any others infected. The cercaria found in the case of each of these snails was identical with the cercaria described here

from naturally infected snails. This experiment is of course open to very obvious objection if it were the only evidence provided, but as purely corroborative evidence it has some value. It is of interest to note that the cercariae from one of these snails were used to infect a monkey and that from this animal the adult male form of *S. haematobium* was recovered.

*C.* Incidence in Snail Host.

*Planorbis* (*Hippentis*) species was found in only one village out of 30 examined, and of 318 specimens dissected none harboured this cercaria, although 151 were infected by cercariae of other kinds.

The snail here found associated with the presence of the disease in human beings was this *Physopsis* sp., nearly akin to *Pl. globosa*, Morelet. This was also the only snail found infected with the cercaria described. The infection rate in these snails with this cercaria was frequently high and was particularly so when the snails were taken in water latrines; in one case 42 per cent. of 50 snails taken in such a site were infected with this cercaria.

*D.* Experimental Infection of Laboratory Animals.

From guinea-pigs and monkeys which were submitted to scanty infection with this cercaria by feeding, adult males morphologically identical with *S. haematobium* were recovered after a period of three months.

Such is the evidence which makes it probable that this cercaria is that of *S. haematobium*.

Reverting now to the differential characters given in Table III it appears that the only one which seemed to be definite has broken down in so far as concerns the cercaria of *S. haematobium* and that we are left with very little upon which to base a diagnosis between the three species of cercariae which affect human beings. The gland arrangement in *mansoni* will doubtless receive further attention and should it be proved from careful study of living specimens that the cercaria of *S. mansoni* possesses five glands on each side, then we shall have reached again the stage at which, pending the production of further morphological facts or other criteria, we must diagnose the cercariae by the experimental method.

BIONOMICS OF *PHYSOPSIS*.

Some observations which appear worthy of recording were made on this snail. The snail was found only in certain situations ; in water which lay or ran slowly on a muddy bottom, where weed or grass grew in the water, and under high or low shade ; it appeared that these three factors—mud, weed, and shade—were essential to it in the water of the localities in which it was found (Pl. XIV, figs. 1 and 2). It was never found in a stream which had a clean sandy bottom, even though weed and overhead shade were present. Further, it was often absent in places which looked at first sight admirable sites for it, in water that is to say with shade, mud and weed ; examination proved that the mud layer at the bottom of the water in these places was only a thin layer lying over sand. This snail likes mud, and the mud-living habit doubtless explains its distribution in the country traversed. In the hilly country, the stream bottoms were covered with sand, and there was no silting of mud. Consequently, this snail was not found at the villages situated round the base of the Loma mountains. Indeed, in the mountainous Koranko country, it was only found on the route traversed at two places. Sokurella and Benikorro, both in relatively low lying country. Its habitat is important from the point of view of prophylaxis against Schistosome infection. It may be mentioned that it was found in large numbers in a wet rice field at Jiama, which was practically marsh land ; on the other hand it was never found in open, quickly flowing streams, in the same locality.

## INFECTION OF *PHYSOPSIS* WITH CERCARIAE OF HUMAN SCHISTOSOMES

Of 1557 specimens of *Physopsis* dissected, 306 had cercariae of some kind present in their tissues. Out of the 306 there were 184 infected with the cercariae of *S. haematobium* which was, as shown above, the only Schistosome affecting the population examined. In some cases where cercariae of *S. haematobium* were found, other cercariae were also present in the same snail. On the other hand, in snails from some localities, little or no infection of any kind was

found ; the variable rate of infection from different localities was a very striking phenomenon. Examples of this are given in Table IV in order of increasing infection.

TABLE IV.

Giving infection rates in *Physopsis*

| Locality | Number of *Physopsis* dissected | Number infected with cercariae of *S. haematobium* | Percentage |
|---|---|---|---|
| Jiama (Nimmi Yemma) ... ... | 497 | 12 | 2·4 |
| Jiama (Nimmi Korro) ... ... | 200 | 18 | 9·0 |
| Bendu ... ... ... ... | 160 | 18 | 11·2 |
| Taiko ... ... ... ... | 48 | 8 | 16·6 |
| Kaiyima ... ... ... ... | 373 | 112 | 30·0 |
| Paya... ... ... ... ... | 50 | 21 | 42·0 |

These high rates of infection in *Physopsis* may be compared with the figures which are given by Manson-Bahr and Fairley (1920) for *Bullinus* in Egypt. These authors discuss the reasons which may account for the fact that while it is difficult to find *Bullinus* infested in any numbers, the infestations of human beings with *S. haematobium* are very numerous. They point out that the highest infestation recorded by them in this snail for one month was 9 per cent., and that it more commonly was 1 or even less, per cent.

On considering the factors which result in the production of a high rate of infection in these snails, we found that the infection rate was directly proportional to the contamination of the water supply in a village by human excreta. The effect of even a small stream, if used for latrine purposes, in producing a high infection of *Physopsis* with cercariae of human Schistosomes is clearly seen on analysing the Jiama (Nimmi Yemma) figures. One section of the village goes to a spring for its drinking water. The path to this spring passes through a shallow, muddy pool into which comes the water from the men's latrine situated a few yards away (see fig. 4). From the spring a stream runs towards the village and turns sharply into a wet rice-field just before reaching the pool mentioned. The spring and the

upper part of the stream from it yielded no *Physopsis ;* the water ran on a bed of coarse sand. Lower down towards the village a layer of mud had formed on the sand and one *Physopsis* was found here. On following this stream into the rice-field where it became

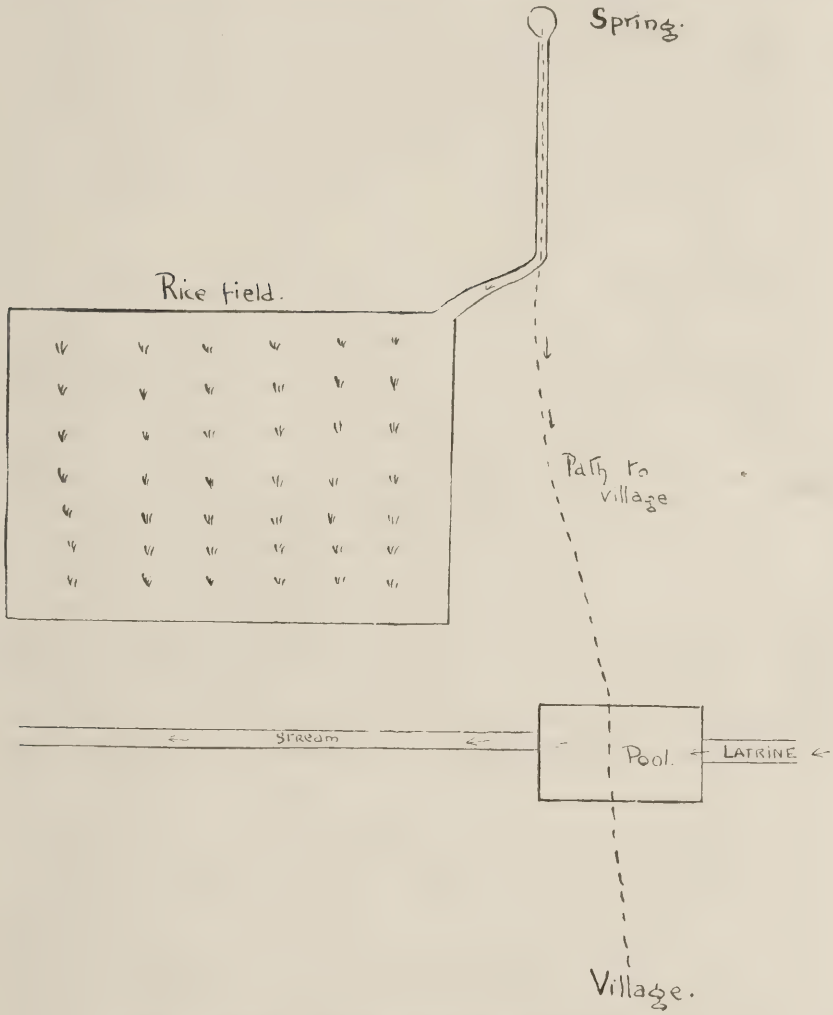


FIG. 4. Diagram of Spring, Rice-field, and Latrine pool at Jiama.

lost in marshy muddy ground, *Physopsis* was easily found, usually on weeds or on the underside of fallen leaves ; many were in the mud and came to the surface after the mud had been trampled down.

In the pool adjacent to the latrine (see Pl. XIV, fig. 1) *Physopsis*

was also found ; the pool water did not enter the rice-field but skirted alongside it for some distance. The result of dissection of snails from these different sources is illuminating, and is set out in Table V.

TABLE V.

Cercariae of *S. haematobium* found on dissection of *Physopsis* at Jiama (Nimmi Yemma).

| Source | Number of *Physopsis* dissected | Number infected | Percentage |
|---|---|---|---|
| Rice field ... ... ... ... | 430 | 1 | 0·2 |
| Stream ... ... ... ... | 1 | 0 | 0·0 |
| Pool adjacent to latrine ... .. | 66 | 11 | 16·6 |

The present position at this corner of the village is that every one in going to fetch water from the spring has to wade through a pool of water which is a veritable sewer and contains highly infected snails. This pool is an ideal one for the dissemination of cercariae since most children in passing through the water play about in it for some time. Situated in such favourable circumstances, the infected snail is able to do the greatest damage to the maximum number of people.

The facts noted here were confirmed almost immediately in the case of two villages in the vicinity, Paya and Taiko ; from each of these villages people had come for treatment. As a return they were asked to collect snails. The instructions given were that they should go to the place where the men's latrine was and search the water there. The results were :—from Paya, 50 *Physopsis* of which 21 that is 42·0 per cent. were infected with cercariae of *S. haematobium ;* from Taiko, 48 *Physopsis* of which 8 that is 16·6 per cent. were similarly infected. A messenger was next sent to Jiama (Nimmi Korro) with identical instructions. He returned 29.12.23 with nearly 300 *Physopsis* of which 200 dissected yielded 18 infected with human cercariae, that is 9 per cent. It is of interest to note that the pool at Jiama (Nimmi Korro) in which these snails were found, dries up in the dry season. A messenger sent there in April found only 6 *Physopsis* alive in the mud at this particular spot.

*Physopsis* then, in addition to being a mud snail here, is also

quite definitely a sewage snail, and that to a very marked degree. It was observed that those *Physopsis* from the water latrines were the largest and most active of all found, as well as being the most heavily infected with human Schistosome cercariae.

The fact that at Jiama the latrine snails were relatively few but heavily infected, while a few yards away the rice field snails were many and scantily infected, is one which may prove of considerable importance from the practical point of view. It is evident that a comparatively simple modification of the latrine arrangements of such villages should result in a great diminution of human Schistosomiasis.

### Resistance of the *Physopsis* sp. to Drying

Experiments were made in order to ascertain to what extent the snails could survive alterations in their environment, especially by drying. The appearance of the snails after comparatively short periods of drying is deceptive ; the animal retracts very far into the shell and looks as if it were dead, but on immersion in water it expands slowly and resumes activity.

#### (1) Alternate Drying and Soaking

5 snails were placed dry in a glass dish in the shade. They were kept thus for a period of 20 hours and then placed in water. After 4 hours in water the whole process was repeated. All 5 snails survived 2 days of such treatment, and 3 survived over 3 days.

#### (2) Drying

2 snails glued on a card were exposed alternately to sun and shade for 48 hours. They were still alive, and moved actively after soaking for an hour-and-a-half in water.

3 snails were tied in dry muslin and placed in the shade. After 3 days they were put in water. 1 was alive. This one was subjected to alternate drying and soaking as in the first experiment. It survived until the 9th day.

5 snails were tied in dry muslin in shade. After 3 days 3 were alive ; dried and tested again on the 5th day, 3 were still alive ; on testing on the 7th day they were dead.

(3) Gradual Drying on Mud

18 snails were placed on wet mud in shade and the water was gradually drained away from the vessel. The snails remained on the surface of the mud adhering to it and retracted within their shells. On the 13th day all the snails were placed in water. After a long period 14 were alive and active. The first showed signs of life only after over an hour's immersion, and the last only after 10 hours immersion.

Depth in Soft Mud attained by the Snails

Many experiments were made to see whether exposure to sun on wet mud or on drying mud would cause the snails to bury themselves. In only one case did a snail go so deep as an inch-and-a-half from the surface, in the vast majority of cases they remained either on, or just under the surface. Direct sunlight was rapidly fatal to snails lying on dry mud.

These experiments prove that the resistance of this snail to various alterations in its environment is by no means negligible. It appears to be a much more resistant snail than *Isodora innesi* of which Archibald (1923) writes: 'if deprived of water for a period longer than six hours, it will surely die.'

The practical importance of the possession of such resistance by this *Physopsis* sp. is that merely cutting off or diverting the water for a few days in infected areas will not ensure the death of all or probably even of many of these snails.

It is of interest to note that in describing the present day conditions of the village of El Marg in Egypt, Faust and Meleney (1924) remark:

'as in the past the water supply was intermittent, being off for five days then on for a period. Snails of the species *Isodora truncata* were collected just north of the village. Leiper's (1915) recommendations have apparently not been carried out.'

One conclusion in the Report to the Government, arrived at as a result of the consideration of the local conditions, was that the proper and only promising methods of dealing with the problem of Schistosomiasis here was firstly by means of attacking the snail where it is known to be infected, and secondly, by so modifying, through education, local customs with regard to water pollution, as finally to prevent infection of snails. Any attempt here at an extensive and wholesale campaign of treatment by antimony or other drugs was deprecated as being, for definite reasons given, likely to defeat its own object and to be an expensive failure.

## SUMMARY

(1) During an investigation into the prevalence of Schistosomiasis in certain districts of the Protectorate of Sierra Leone, infection due to *S. haematobium* was the only type of the disease found.

(2) *Physopsis* c.f. *globosa*, Morelet, was proved to be the intermediate host; the infection rate in the mollusc with cercariae of *S. haematobium* was often very high, *e.g.*, 42 per cent. in a water latrine.

(3) A description is given of the morphology of the cercaria of *S. haematobium*; it differs markedly from the description of this cercaria at present accepted.

(4) A critical analysis of the basis of the existing description is undertaken.

(5) Some facts relating to the bionomics of *Physopsis* c.f. *globosa* are mentioned; experiments showed that this snail was resistant to drying to an unexpected degree.

(6) Of snails placed on mud in the shade a large percentage survived for a fortnight when the water was drained away gradually.

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## EXPLANATION OF PLATE XIV

Fig. 1. View of latrine pool at Jiama. (*Vide* fig. 4). Infected *Physopsis* found.

Fig. 2. View of washing and latrine pool at Kaiyema. Infected *Physopsis* found.

Fig. 3. View of men's bathing place on the River Kaiso. No snails found. Sandy bottom, no shade, quickly flowing.

FIG. 2

FIG. 1

FIG. 3

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# OBSERVATIONS ON THE CLASSIFICATION OF CERTAIN SCHISTOSOME CERCARIAE

BY

B. BLACKLOCK, M.D.

AND

M. G. THOMPSON, M.B., B.Sc.

(*From The Sir Alfred Jones Research Laboratory, Freetown*)

(*Received for publication*, 23 *June*, 1924)

Since sending our description of the cercaria of *S. haematobium* to press, we have had the opportunity of seeing Khalil's paper on the cercaria of *S. mansoni* and Bettencourt and da Silva's paper on the cercaria of *S. haematobium* in Portugal; Khalil (1922) gives an account of the morphology of the cercaria of *S. mansoni* from *Planorbis boissyi* of Egypt. We find that his description of this cercaria corresponds almost in every detail with our description of the cercaria of *S. haematobium*. He describes, however, four oral glands with four ducts, but he qualifies this by the statement 'distinct outlines of separate glands cannot be made out' and that their duct openings are 'apparently unarmed with papillae.' He also describes two ciliated areas in the beginning and end of the main excretory duct. We referred in our description to our difficulty in making these structures out in the cercaria of *S. haematobium* and we also mentioned the variable accounts of these structures given by different authors. Bettencourt and da Silva (1922) found such areas in two fusiform dilatations in the tubes of the excretory system of the cercaria of *S. haematobium*, but noted that they were not easily observable in all specimens, and as a rule were not visible except when the cercaria was on the point of death. These ciliated areas are evidently so difficult to locate that they do not afford sufficiently reliable information for purposes of classification.

In Khalil's paper, describing the cercaria of *S. mansoni* from *P. boissyi*, the second figure in the text is labelled 'Camera lucida

drawing of *Schistosomum haematobium* cercaria.' This we conclude is a mistake for *S. mansoni*, for the following reasons :—

1. The title of the paper is ' Cercaria of *Schistosomum mansoni*.'
2. The material studied (p. 1) is from *Planorbis boissyi*.
3. The text is entirely a description of *S. mansoni* cercariae.
4. Khalil states (p. 5) : ' There is no reliable account of the anatomy of *Schistosomum haematobium* cercariae available.'
5. There is no figure of the general anatomy of the cercaria of *S. mansoni* except this, which is labelled *Schistosomum haematobium*.

Bettencourt and da Silva describe the cercaria of *S. haematobium* as possessing only three pairs of unicellular glands in the posterior region of the body ; the cells are of one type, having their protoplasm filled with coarse acidophilic granules. Three ducts lead forward from these and end in the openings. Our description of the cercaria of *S. haematobium* is at variance with that of Bettencourt and da Silva.

A study of this more recent work does not assist us in differentiating the cercaria of *S. haematobium* as described by us from that of *S. mansoni* as described by Khalil, by means of the morphology ; further, it does not assist us in distinguishing either of these from *Cercaria indica* XXX described by Sewell. By what means can we distinguish the three human Schistosome cercariae, *japonicum*, *haematobium*, and *mansoni* from each other and from *Cercaria indica* XXX ?

Morphology. If the secretory glands of *japonicum* are all of one type under all conditions and in all specimens, we have here a character to distinguish it from the other three. The most recent studies on the cercariae of *S. haematobium*, *S. mansoni*, and *C. indica* XXX disclose no constant morphological character nor staining reaction by which they can be distinguished one from the other.

## CLASSIFICATION BY MEANS OF THE IMMEDIATE HOST

As our knowledge stands at present, classification of the above four Schistosome cercariae by means of mollusc hosts does not appear possible ; but a critical survey of observations on this point is interesting. Observers, however, are somewhat handicapped by the uncertainty and changeable nature of mollusc nomenclature. Annandale (1924) states that hitherto the cercariae of *S. japonicum*

have been found only in molluscs of the genus *Oncomelania*: 'Probably all the living species of the genus are potential carriers of *S. japonicum* but further information is needed on this head.' He states also that there is no evidence that *Blandfordia* is a disease carrier. Up to the present then, the cercaria of *S. japonicum* has only been found in the genus *Oncomelania*—but we do not know whether it will develop in *Planorbis* or *Bullinus*, nor yet whether *mansoni* or *haematobium* will develop in *Oncomelania*. Bettencourt and da Silva describe *haematobium* cercariae from *Planorbis* in Portugal. This observation appears to upset the possibility of classification by snail host. With regard to *S. haematobium* and *S. mansoni*, Leiper (1915) found that: 'In females reared from *Bullinus* the eggs are constantly terminal-spined, even in small young females. . . . In females reared from *Planorbis boissyi*, the eggs are constantly lateral-spined.' The work of Faust, Porter and the Portuguese observers, together with certain facts from India appear to show that classification by snail host is not now reliable; yet it seems remarkable that in Egypt where there are both forms of these human miracidia present and snails of both *Bullinus* and *Planorbis* genera, there has never been reported, so far as we are aware, any deviation from this selective habit. The geographical distribution of the infections does not suggest that the genus *Planorbis* can be generally utilized to propagate *haematobium*.

Before we can definitely state that classification by the snail host must be discarded, it appears to us essential that very complete experimental evidence must be brought forward which will associate clearly the cercariae from any supposed snail host with the adult worm. While the classification of the cercariae belonging to *S. haematobium* or *S. mansoni* appears to us impossible on the grounds of morphology or staining reactions, we are not in a position to state at present whether the snail host will finally help us in this matter.

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# MISCELLANEA

## LARVA OF THE TUMBU-FLY *CORDYLOBIA ANTHROPOPHAGA* IN THE LOWER EYELID

PLATE XV

This case was shown to me by Dr. Wright of Freetown, under whose care it was. The larva, about one-third grown, had been removed some time before the photograph was taken and the swelling had largely subsided.

The Sir Alfred Lewis Jones Research Laboratory,
11.6.24.

B. BLACKLOCK, M.D.

## THE HATCHING *IN VITRO* OF THE EGGS OF *OXYURIS EQUI*

It is well known that the embryonated eggs of *Oxyuris equi* occasionally hatch in saline solution, but the phenomenon is an inconstant one, and Schwartz (1923) who has recently studied it, concludes that it is purely accidental. The larvae which emerge are usually motionless or show only feeble or transient activity, and as pointed out by Schwartz they often are nipped by the too narrow opening in the egg shell and fail to emerge completely. It would seem that this phenomenon is capable of a purely mechanical explanation, for we have observed it in the case of eggs which had been kept dry at a temperature of 27° C. for over seven months, in which the embryos, although well preserved, showed no signs of life. Some experiments were, therefore, carried out to determine if more

satisfactory results could be obtained with media, approximating in some respects more closely to those which, if the eggs were ingested, would be met with in the alimentary canal.

The eggs used were collected from the margin of the anus of a horse and contained active embryos: at the time when the experiments were made they had been kept dry, at a temperature of 27° C., for from one to two weeks. It was found that immersion of such eggs in an acid solution of pepsin for twenty minutes did not cause them to hatch, but that if they were then transferred to either a solution of pancreatic juice or to a saline solution of approximately equal alkalinity, a large number of active larvae emerged. As the result of further experiments, of which that given in the table is an example, it was found that immersion of the eggs first in an acid solution (Hydrochloric acid, 0.1 to 0.2 per cent.) for half-an-hour or longer, and then in an alkaline solution (Caustic Potash or Sodium Carbonate from 0·1 to 0·5 per cent.) invariably caused large numbers of active larvae to hatch, whereas the acid solution alone and the alkaline solution alone failed to produce this result. On comparing the action of an acid pepsin solution with that of a corresponding solution of acid alone, little difference was observed, but in one or two experiments the number of eggs which hatched in the former was rather greater.

Experiment to test the action of acid followed by alkali on eggs of *Oxyuris equi*.

| Time | Normal saline solution | | Hydrochloric acid, 0·1 per cent. | |
|---|---|---|---|---|
| 5·15 p.m. | Experiment started | | Experiment started | |
| 5.45 p.m. | No free larvae | Some of the eggs transferred to 0·5 per cent. sodium carbonate solution. | No free larvae. | Some of the eggs transferred to 0·5 per cent. sodium carbonate solution. |
| 6.10 p.m. | No free larvae | No free larvae | No free larvae | No free larvae |
| 6.50 p.m. | No free larvae | No free larvae | No free larvae | A few larvae have hatched, most of them actively motile |
| 8.10 p.m. | One free larva; appears dead | One free larva; appears dead | No free larvae | Very many larvae have hatched |

R. M. Gordon and J. W. S. Macfie.

The following parasites have been identified from animals which died at Sierra Leone.

*Taenia taeniaeformis* (Batsch, 1786) Wolffhügel, 1911.

Synonym : *T. crassicollis*, Rud., 1810.

Numerous specimens from domestic cats. The large hooks measured 385$\mu$ in length and the small hooks 225$\mu$.

*Cysticercus fasciolaris*, Rud., 1808.

Two specimens from the liver of a rat.

*Dipylidium caninum* (Linn. 1758) Rail. 1892.

Four specimens from a civet cat and numerous specimens from dogs.

*Hymenolepis diminuta* (Rud., 1819) R. Blanchard, 1891.

Numerous specimens from rats.

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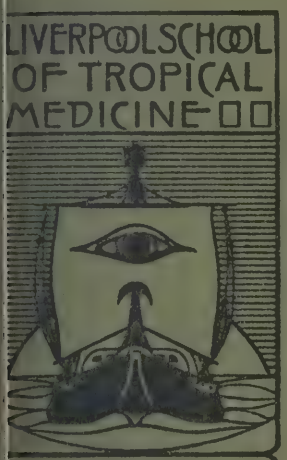


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OF

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SPECIMEN PAGE



*The nymph or pupa* (fig. 7). This lies with its head towards the anterior pole of the puparium, and its venter usually on the side of the puparium with the greatest convexity. It is somewhat ovate and almost fills the whole of the interior of the puparium. The sheaths of legs II and III are curved over the end of the abdomen, but the tip of the rostrum does not reach the end of the tarsi of the front pair of legs.

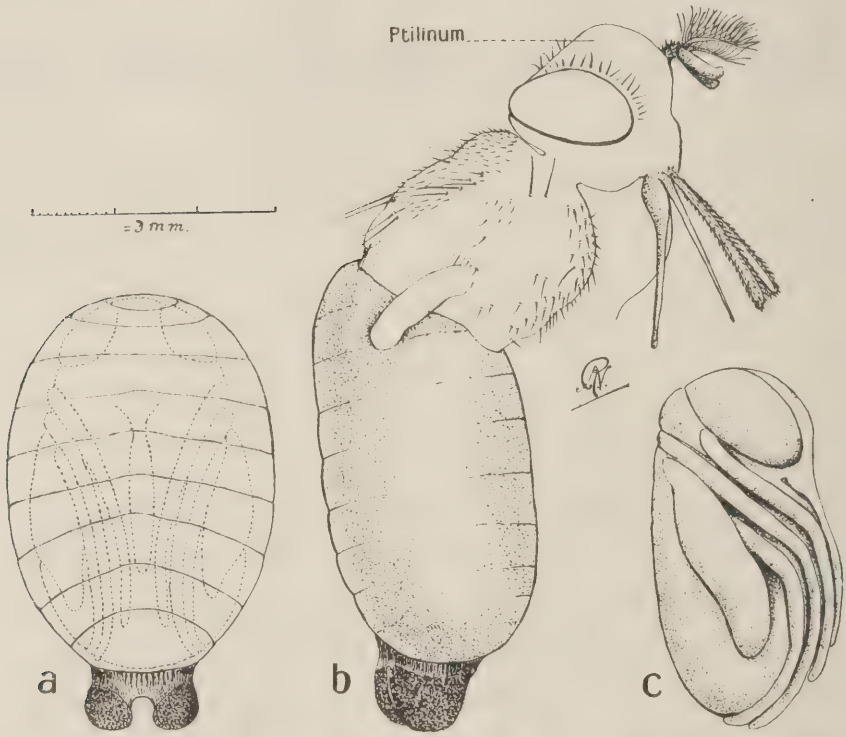


FIG. 7. *Glossina morsitans.* *a*—puparium with pupa (dotted lines) *in situ*; partly diagrammatic; *b*—tsetse-fly emerging from puparium, showing frontal sac (ptilinum), which the fly uses to break off the anterior end of the puparium and also to force its way through the earth or humus; *c*—pupa removed from the puparium. *b* and *c* were drawn from examples restored in caustic potash.

The imago escapes by pushing off the end of the puparium by means of its large frontal sac or ptilinum (fig. 7, *b*), placed in front of the head between the eyes; this the insect inflates at will. This sac is also used to force a passage through the superincumbent earth or humus; but on reaching the open air, it is permanently withdrawn and is rendered functionless.

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# ON THE ASCARIDS OF THE DOG AND CAT

BY

E. L. TAYLOR, M.R.C.V.S.

*From the Parasitological Laboratory of the Liverpool School of Tropical Medicine.*

(*Received for publication* 9 *June*, 1924)

On looking through the collection of the *Ascarids* of the dog and cat at the Liverpool School of Tropical Medicine, four of the several specimen jars of worms from the cat were found to contain a species of *Toxascaris ;* of these, two contained *Toxascaris* sp. together with *Belascaris mystax*, while the remaining two contained *Toxascaris* sp. only. As no species of the genus *Toxascaris* seems to have been previously recorded* in the cat, the specimens were examined more closely and were found to present the following specific characters : Length of male 19 to 65 mm., length of female 22 to 80 mm. Cuticle finely striated, the striations being a distance of 4 to 9$\mu$ apart. The labial pulp shows two anterior lobules, detached from the main pulp by a well-marked cleft, and presenting a shallow depression at their extremities. The cervical alae are long and narrow, gradually decreasing in width posteriorly.

The caudal extremity of the female terminates in an acute point, and the vulva is situated about the junction of the anterior and middle thirds of the body. The eggs are 70 to 80$\mu$ in diameter, globular or subglobular and have a thick, smooth shell. The caudal extremity of the male presents a number of papillae of which there are six post anal on either side, two sub-dorsal, one lateral, small and difficult to see, and two sub-ventral, while just behind the anus on either side is a large double papilla ; these last three may be regarded as a continuation of the row of preanal papillae. Anterior to the anus on either side is a row of twenty-five or more preanal

* Since writing this paper my attention has been drawn to the fact that Baylis (1924), has recently recorded the presence of *Toxascaris leonina* in the cat.

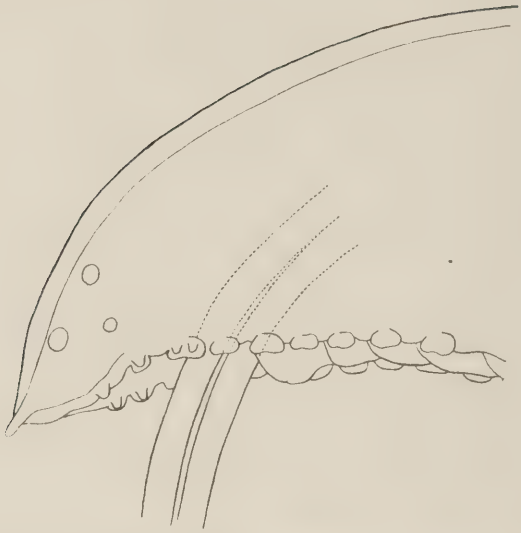

FIG. 1A. *Toxascaris*, sp. Cat. Caudal extremity, lateral view.

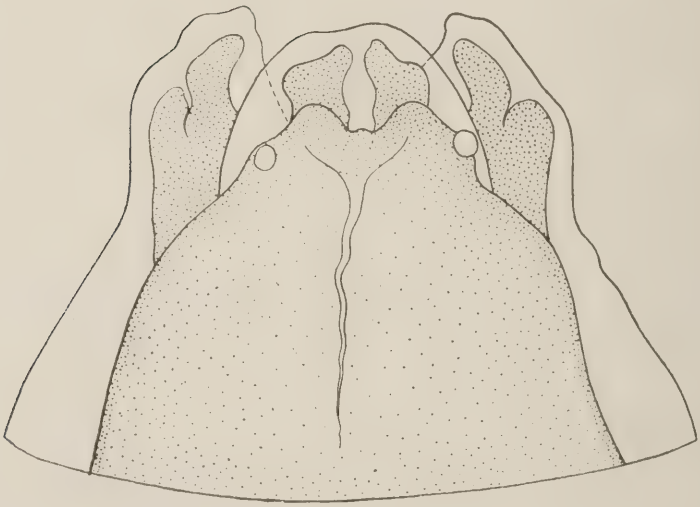

FIG. 1B. *Toxascaris limbata.* Head, dorsal view.

papillae, sub-ventrally placed; these increase in size posteriorly and towards the anus are large and can be much more readily distinguished than in the male *Belascaris mystax*.

The spicules of the male are 0·7 to 1·26 mm. in length and slightly unequal; they are not winged and are extended in about 25 per cent. of preserved specimens.

The lengths and other details of the worms belonging to the genus *Toxascaris*, collected from the four cats, are given in Table I.

*Toxascaris limbata* from the dog was now examined with a view to comparison, and measurements were made of the worms collected from four dogs. No difference in general morphology and microscopic appearance could be found. Details of measurements of these worms are given in Table II and it will be noticed in comparing these two tables that while the individuals vary greatly in size, there is no general difference between those from the two hosts.

Specimens of *Toxascaris leonina* from the lion were now examined, and, as with *Toxascaris limbata*, were found to differ in no particular from the species found in the cat. Details of measurements are given in Table III.

Railliet and Henry (1911) describe three species of *Toxascaris*, viz., *T. leonina*, *T. limbata* and *T. microptera*. Of the last species only two poor specimens were available and the description given is therefore very incomplete. *T. limbata* and *T. leonina* are described more fully and both descriptions seem to tally very well with the species of *Toxascaris* from the cat. Measurements of the two species made by Railliet and Henry are given in Table IV, and it will be seen that differences in size are very slight. The only other distinguishing features mentioned by these two observers are, firstly, that the caudal extremity of the female *T. limbata* terminates in a more acute point; and secondly, that the spicules in the male *T. leonina* are more frequently extended. These two relative characters seem to be of little value in distinguishing the two species. The comparative sharpness of the caudal extremity in *T. limbata* I have not been able to see, while the relative frequency with which the preserved male worm is found to have the spicules retained within the body or protruding from it seems to be a variable factor, since observations on a large number of specimens of *Belascaris mystax* and *Belascaris marginata* at my disposal, have shown the

spicules to be more frequently retained in the former than in the latter species, whereas Railliet and Henry found the reverse.

In my opinion, there is insufficient reason to warrant the division of the genus *Toxascaris* into the three species mentioned, and the

FIG. 2A. *Belascaris mystax.* Caudal extremity, lateral view.

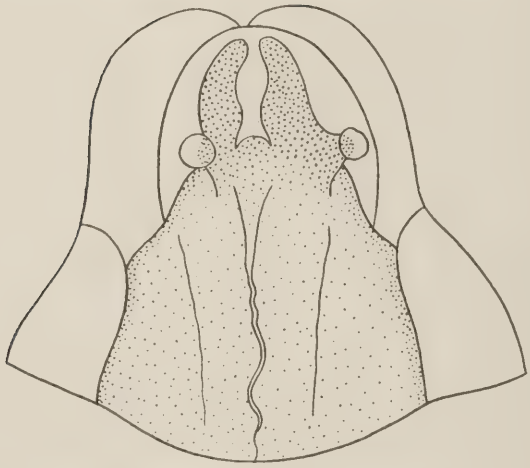

FIG. 2B. *Belascaris mystax.* Head, dorsal view.

Toxascarids of the lion, the dog and the cat are probably identical, and should be known as *Toxascaris leonina* (Linstow, 1902). To the three hosts mentioned, may be added the various hosts of

*T. leonina* given by Baylis and Daubney (1922), the tiger, leopard, ounce or snow leopard, fishing cat, leopard cat, hunting leopard and Indian fox.

Worms of the genus *Belascaris* from the dog and cat were found to fall into two distinct and definite species, viz., *Belascaris mystax*, confined to the cat, and *Belascaris marginata* confined to the dog. The most striking difference between these two species is seen in the spicules, which are relatively much larger in *B. mystax*, being about one twenty-fifth of the body length, while in *B. marginata* they are only one seventy-fifth of the body length. The difference between the cervical alae is also well marked ; in *B. mystax* they are broad, having their widest part near their posterior extremities, where they terminate abruptly ; while in *B. marginata* they are long and narrow and terminate gradually, resembling those of *Toxascaris leonina*. Particulars of measurements from numbers of these worms are given in Tables V and VI.

FIG. 3A.
Cervical aleae.—*Belascaris mystax.*

FIG. 3B.
Cervical aleae.—*Toxascaris limbata.*

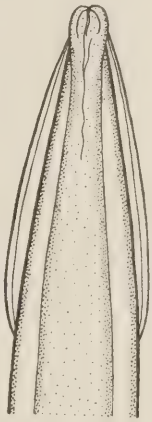

FIG. 3C.
Cervical aleae.—*Belascaris marginata.*

I have not been able to satisfy myself as to the presence of the caudal alae mentioned by Railliet and Henry (1911) as very distinct in *B. mystax*. Several specimens when rolled under the microscope presented a dark longitudinal line on either side resembling the edge of a wing, turned upwards ; but on examining the same caudal extremities in section, the effect was seen to have been produced by the dark, somewhat opaque walls of the much flattened gut, which, seen on edge, through the cleared cuticle of the specimen might be mistaken for caudal alae,

TABLE I

Showing measurements of *Toxascaris* sp. from the cat.

| Specimen bottle | Length of male in mms. | Length of female in mms. | Length of spicules in mms. | Distance between striations in $\mu$ | Size of eggs in $\mu$ |
|---|---|---|---|---|---|
| No. I ... ... | 65 | 80 | 1·26 | 9 | ... |
| No. II... ... | 40-50 | 47-75 | 0·80-0·95 | 7 | 60 × 81 |
| No. III ... | 19-25 | 22-23 | 0·70-0·87 | 4-5 | ... |
| No. IV ... | 32 | 30 | 0·89 | 7-8 | ... |

TABLE II

Showing measurements of *Toxascaris limbata* from the dog.

| Specimen bottle | Length of male in mms. | Length of female in mms. | Length of spicules in mms. | Distance between striations in $\mu$ | Size of eggs in $\mu$ |
|---|---|---|---|---|---|
| No. I ... ... | 30-35 | 40-60 | 0·95-1·04 | 5 | 60 × 75 |
| No. II... ... | 30-48 | 65-80 | 0·89-1·04 | 8·5 | ... |
| No. III ... | 40-45 | *(18)40-60 | Old specimens could not be clearly seen | 7·5 | ... |
| No. IV ... | 55-70 | 75-100 | 1·11-1·27 | 7·4-14·6 | 74 × 86 |

*This is the measurement of one very small worm among a number of larger ones.

TABLE III

Showing measurements of *Toxascaris leonina* from the lion.

| Specimen bottle | Length of male in mms. | Length of female in mms. | Length of spicules in mms. | Distance between striations in $\mu$ | Size of eggs in $\mu$ |
|---|---|---|---|---|---|
| No. I ... ... | 37 | 50-68 | 1·12 | 8-9 | 60 × 85 |
| No. II ... | 28-52 | 29-63 | 1·00-1·50 | 4-10 | 66 × 75 to 70 × 84 |
| No. III ... | 37-43 | 39-70 | 1·05-1·20 | 4-11 | 66 × 75 to 68 × 84 |

TABLE IV

Showing the variation of length and other detailed measurements in the *Toxascaris* spp. from the cat, dog and lion, together with the measurements of *T. leonina* and *T. limbata* given by Railliet and Henry.

| | Length of male in mms. | Length of female in mms. | Length of spicules in mms. | Distance between striations in $\mu$ | Size of eggs in $\mu$ |
|---|---|---|---|---|---|
| *Toxascaris* sp. from the cat | 19-65 | 22-80 | 0·7-1·26 | 4-9 | 60 × 81 |
| *Toxascaris limbata* from the dog | 30-70 | 40-100 | 0·89-1·27 | 5-14·6 | 60-74 to 75-86 |
| *Toxascaris leonina* from the lion | 28-52 | 29-70 | 1·00-1·50 | 4-11 | 66 × 75 to 70 × 84 |
| *Toxascaris leonina* [Railliet and Henry] ... | 20-50 | 30-80 | 0·9-1·25 | 5-8 | 70-80 |
| *Toxascaris limbata* [Railliet and Henry] ... | 40-60 | 65-100 | 1·2-1·5 | 6-12 | 75-85 |

TABLE V

Showing measurements of *Belascaris marginata* from the dog.

| Specimen bottle | Length of male in mms. | Length of female in mms. | Distance between striations in $\mu$ | Length of spicules in mms. |
|---|---|---|---|---|
| No. I ... ... ... | 70-90 | 90-165 | 13-37 | 0·95 (extended) |
| No. II ... ... | 70 | 67-170 | 12-24 | 1·05 (retained) |
| No. III ... ... | 70 | 120-130 | 18-24 | 1·01 (extended) |
| No. IV ... ... | 55 | 80-90 | 13·6-24 | 1·04 (extended) |
| No. V ... ... ... | 50-75 | 50-120 | 9-27 | 0·74-1·3 (varying positions) |

TABLE VI

Showing measurements of *Belascaris mystax* from the cat.

| Specimen bottle | Length of male in mms. | Length of female in mms. | Distance between striations in $\mu$ | Length of spicules in mms. |
|---|---|---|---|---|
| No. I ... ... ... | 60 | 85-108 | 24-37 | 1·98 (retained) |
| No. II ... ... | 27-40 | 42-70 | 14·5-25 | 1·85-2·08 (varying positions) |
| No. III ... ... | 53 | 80 | 18·5-28 | 2·08 (retained) |
| No. IV ... ... | ... | 63-85 | 18·5-29 | ... |
| No. V ... ... ... | 42-70 | 90-105 | 10-27 | 1·63 (retained) |

TABLE VII

Showing the variations in length and other details in *Belascaris marginata* and *Belascaris mystax*, together with those given by Railliet and Henry.

| | Length of male in mms. | Length of female in mms. | Distance between striations in $\mu$ | Length of spicules in mms. |
|---|---|---|---|---|
| *Belascaris marginata* from the dog ... | 50-90 | 50-170 | 9-37 | 0·74-1·05 (usually extended) |
| *Belascaris mystax* from the cat ... | 27-70 | 42-108 | 10-37 | 1·63-2·08 (usually retained) |
| *Belascaris marginata* (Railliet & Henry) ... | 50-100 | 90-180 | 16-22 | 0·75-0·95 (rarely extended) |
| *Belascaris mystax* (Railliet & Henry) ... | 30-60 | 40-100 | 12-16 | 1·7-1·9 (generally extended) |

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# CRAW-CRAW IN SIERRA LEONE

BY

B. BLACKLOCK, M.D.

*From the Sir Alfred Lewis Jones Research Laboratory, Freetown*

(*Received for publication* 23 *June*, 1924)

PLATES XVI and XVII

Of all diseases seen in Sierra Leone natives, both in the Colony and Protectorate, none is more widely distributed and few are more productive of physical discomfort and chronic skin lesions than the disease called Craw-Craw in the Creole *patois* of the Liberated Africans. This disease has long been the subject of much speculation. Clarke (1848) who was Senior Assistant Surgeon to the Colony of Sierra Leone referred to it as Kru-Kru and it is here that we see the origin of the name. The description of the condition given by Clarke is :—

'KRU-KRU. This disease is characterised by an eruption which first appears in small pustules between the fingers, on the wrists, arms, hams, legs, and feet ; these pustules becoming confluent are ultimately spread over the body. It, however, sometimes occurs in separate patches on the neck, breast, back and hips, etc., when it is known among the Liberated Africans by the term Krooman's Kru-Kru. The itching and irritability are very great, which might be expected in a climate where so many debilitating causes exist. Yaws are often associated with this complaint and it is worthy of observation that leprotic disease not unfrequently follows a severe attack of Yaws. Kru-Kru occasionally occurs, in the persons of Europeans.'

Many years later O'Neill (1875) says of Craw-Craw :

'At first sight a well-marked case suggests the presence of extensive scabies in all its stages of development—the papule, the vesicle, and the pustule ; showing itself first in the clefts of the fingers, front of the wrists, and back of the elbows, seldom being found on the face, and always accompanied with intense itching. It should be stated that all the cases examined, about six in number, were in the persons of negroes, where the native hue of their complexion obscures the blush which, in the case of a "white man" would surround every eruption accompanied with irritation. The papules arise singly and at irregular intervals, increase to the size of a pin's head, feel firm to the touch, and, on account of the reason above stated, appear of the same colour as the surrounding integument. In some cases the papules arrange themselves in a crescentic form, like ringworm ; still it appears this is accidental, and that the separate and scattered distribution is the more common. In about two days' time the papule becomes converted into the vesicle, with very little increase in size, and in the course of a couple of days the pustule

is developed, rapidly enlarging, and uniting with those in its immediate neighbourhood. In the height of his suffering the patient tears the pustules, and their liberated and desiccated contents produce large and unsightly crusts. Night or day produces little alteration in the amount of itching, the cool of night tending, if anything, to lessen the irritation. The contagiousness of this disease is so well known that those affected are most studiously avoided. Three days' time is said to be the period at which the complaint shows itself after productive contact and it is popularly believed that, though a person affected should, in search of its riddance, proceed to the Cape of Good Hope or some other cool latitude, the disease which thus merely becomes latent will burst out with all its former vigour when the unfortunate patient returns to the warmth of the tropics. Sulphur, so beneficial in scabies, is here of doubtful efficacy, and the nostrums of the native "medical man" have frequently failed in bringing relief after six months' application.'

Kennan (1909) examined cases of Craw-Craw in Sierra Leone and in the Gold Coast Colony, and concluded that the disease, acute Craw-Craw, had an onset resembling that of an acute exanthem with a temperature at the earliest stage of 101-102° F. associated with definite constitutional symptoms—vertigo, headache, malaise. The rash which was vesiculo-papular in the earliest stages might be practically universal, but most commonly affected the exterior aspects of the arms and legs. Itching was not a prominent symptom in his cases and was commonly absent, especially in the earlier stages; when present later it usually disappeared before the rash had gone. He noted that all the cases seen were amongst natives and that they were mostly adult young men, not always of the poorest or dirtiest class. Kennan, as a result of his observations, concluded that the disease was contagious and that many cases are overlooked or diagnosed as scabies, etc.

Castellani and Chalmers (1919) say:

'Under the term Craw-Craw, African natives denote practically any pruriginous skin disease. Our African experience has taught us that most of the so-called Craw-Craw cases are cases of neglected scabies or of tinea corporis, or what Daniels and ourselves call cooly itch. *We apply the term Craw-Craw to a dermatosis met with in Africa, in Ceylon, and in various parts of the tropics, characterised by the presence of numerous hard, almost horny papules occasionally slightly exfoliating at the top, varying in size between a millet seed and a small pea. Some of the papules may be follicular. They are not of constant shape; some may be roundish and flattened, and others acuminate.*' [Italics not in original.]

Macleod (1920) says:

'Craw-Craw is a generic name which has been applied indiscriminately in West Africa both by white men and natives to various skin affections generally characterised by itching and pustulation. In 1875 O'Neill described under this heading a vesico-pustular affection which suggested scabies in its clinical characters and distribution. According to Bennett the uneducated natives of Calabar

employed the name for practically all skin diseases, while the more intelligent natives limited its application to three conditions, namely leprosy or bad Craw-Craw, Tinea circinata or Krooboy's Craw-Craw, and Craw-Craw proper, a papulo-vesicular disease which he believed to be pustular eczema. Under the same heading Plehn has described among the natives on the Cameroon coast a papular dermatitis which chiefly attacks the inside of the thighs. Emily has used the name in the French Congo for a chronic pustular disease which began as a reddish brown spot, was excoriated by scratching, and transformed into a superficial ulcer the floor of which gradually became covered with pale granulations secreting thick tenacious pus.

'The above are a few examples of the wide use of the term, but there are many others and except as an interesting native name it might well be abandoned.'

Manson's Tropical Diseases (1921) states that :

'the hard horny papules of craw-craw have to be differentiated from scabies, which is common in African natives.'

An opportunity was found in 1923-4 for observing large numbers of cases of Craw-Craw during an expedition through the Protectorate of Sierra Leone. The native names of several diseases are given below and it will be seen that Craw-Craw is distinguished in all these native tongues from leprosy, small-pox, and yaws. Each of these diseases is recognised clearly and the signs and symptoms are well known to the natives. The Creole word popularly used for small-pox is of some interest, 'Big Daddy,' *i.e.*, the old man, a term of great respect, denoting the legend by which the disease is associated, with the apparition of an aged man in the early epidemics.

Table I

| Native Language | Name of Disease | | | |
|---|---|---|---|---|
| | Leprosy | Yaws | Small-pox | Scabies |
| Creole ... ... | Leprosy | Yaws | Big Daddy | Craw-craw |
| Temne ... ... | Arom | Katiri | Kabumbo | Tabool |
| Foulah ... ... | Barashi | Sareh | Badeh | Pohyeh |
| Mendi ... ... | Kpokpoli | Kewei | Bomboi | Nohoi |
| Limba ... ... | Teemo | Bongbo | Sambeh | Mootaki |
| Susu... ... ... | | Suti | Senyak | Kasi |

Those who were suffering from Craw-Craw in the villages were examined: the skin lesions produced for inspection by persons who stated that they were suffering from the disease were very varied and often severe, especially when the disease had been of long duration. In adults the most chronic and severe effects were seen in the gluteal and genital regions, in children carried on the mother's back the skin of the chest, abdomen and thighs were most affected; in older children the finger clefts, hands and arms were the commonest sites. The only regions where the disease was not often seen were the head, palms and sole of the feet. No matter how old the infection, and notwithstanding the multiplicity of lesions of very diverse character which existed at the time of examination in the various parts of the body affected, there were factors common to all cases examined.

(1) The disease was characterised by itchiness.

(2) The sufferer, on being asked to point out the source of the trouble, invariably pointed to a minute papule or a vesicle which contained clear and rather viscid fluid.

(3) The small pustules frequently seen near the vesicles were considered no longer to contain the source of the disease, because scratched.

(4) The papules and vesicles which appeared to have an unbroken surface, were always found, with the hand-lens, to have a minute opening, usually central in position.

Especially in the gluteal region great thickening of the skin with the production of cicatrices, crusts and scales as a result of continual scratching and secondary infection was the rule. The armpit frequently presented a similar condition. But in small islands of healthy-looking skin the same small papules and vesicles would be indicated as the origin of the disease.

When the disease was fairly generalised over the body and of recent origin, the patient often had malaise and explained that he felt sick, that his stomach was out of order and that he had fever and headache. This condition was to some extent analogous to that described by Kennan as 'acute Craw-Craw' except that in these cases, itching of the papules and vesicles was constantly complained about.

## METHODS OF EXAMINATION USED

All that could be seen in the recent papule and vesicle was a small aperture surrounded by minute scales of dried epidermis. Dissecting out with needles was tried in the case of the fingers, the hand being placed on the stage of the dissecting microscope. In this way the parasite was discovered on several occasions, while on other occasions its eggs were found, and also the immature stages. In morphology the parasite found was not distinguishable from *Sarcoptes scabiei.*

The difficulty attending the discovery of the acarine of scabies in black skins has been referred to by Carlyle-Johnstone (1924).

In reviewing the Biology and Pathology of the natives of the Central Kavirondo District, Kenya Colony, he says :—

> 'Scabies, from which large numbers of the children suffer and which is not uncommon in adults, causes quite a lot of disability. As a result of scratching septic conditions often supervene. It is interesting to note that the causative organism of this disease has not yet been isolated. The cases were diagnosed as scabies on clinical grounds alone and they responded to ordinary sulphur treatment.'

## CRAW-CRAW OR SCABIES IN SIERRA LEONE

The conclusion reached as the result of studying the history, and careful physical examination including the use of the hand-lens, was that the disease called Craw-Craw by the Sierra Leone Creole and the disease of which the various native names were translated as Craw-Craw is none other than scabies. The discovery of the parasite in many typical lesions of Craw-Craw cases confirmed this. It is usually assumed that the parasite of scabies is easily found. In my experience this is not so even in the white skin ; in the black skin the discovery of it is by no means easily made ; those who distinguish Craw-Craw from scabies state that the two conditions can be separated from each other since in Craw-Craw the mite is not found. It is not necessary to consider such conditions as that called Craw-Craw by Castellani and Chalmers. It seems rather like piracy to describe for Africa and Ceylon a new disease and use the name Craw-Craw for it in quite an arbitrary fashion. Craw-Craw, after all, is the Sierra Leone name of a disease which occurs in Sierra Leone and a very definite disease at that.

Its main characters are that it is a contagious disease which may affect persons of any age, and which takes the form of a definite eruption on the skin. It may be localised or generalised and any part of the body may be affected ; with the exceptions referred to above. It may have an acute onset and produce in its early stages definite constitutional symptoms depending upon the intensity of the infestation. The early lesion is a minute papule with an opening which is visible only by the use of a lens ; this is followed by a vesicle and this in turn by a pustule. The greyish burrow characteristic of scabies in the white skin is not observed in the black skin, nor is the faecal discolouration observed. This renders the diagnosis less obvious. If treated efficiently the disease stops at any stage so far described. But owing to non-recognition of the cause of the disease and to the appropriate treatment not being applied, the disease is allowed to assume a chronic form in which it is maintained by severe scratching ; this process assists not only in spreading the infection to healthy skin, but also produces from sepsis serious lesions in the affected area. Thus one sees in cases of some years standing affecting the gluteal and genital regions a very remarkable appearance. The skin is fissured and cracked, and covered with cicatrices and horny papules and ridges, an eczematous condition is frequently seen and ulcers of varying size. At this stage cases are difficult to treat and are often intractable because the skin condition is such that efficient treatment cannot well be tolerated.

Thus Craw-Craw here presents characters which are common to it and scabies as seen in England, but it presents additional features which result from prolonged and neglected infection, and which are seldom seen in England.

The recognition of the fact that Craw-Craw here is scabies is important, because as for scabies everywhere, early treatment of a somewhat drastic kind is essential for its eradication.

In the course of examination of patients for Craw-Craw, I found, as previous observers have done, cases in which the skin in the papules was infected with nematode larvae. These larvae are not the cause of Craw-Craw as they are frequently found in parts of the body which are not affected with Craw-Craw, and, furthermore, they are found in the normal looking skin of some persons who

are not suffering from Craw-Craw; they appear in these cases to give rise to no local or general symptoms by their presence.

## SUMMARY

1. The disease called Craw-Craw in Sierra Leone is identical with scabies.

2. When the eruption is generalised and of recent origin, it sometimes corresponds to the condition described by Kennan as 'acute Craw-Craw.'

3. The parasite which gives rise to it is identical morphologically with *Sarcoptes scabiei*.

4. The parasite is frequently very difficult to discover in the black skin.

5. From long persistence in an untreated condition the disease often causes severe lesions in the skin.

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## EXPLANATION OF PLATE XVI

FIG. 1. Craw-Craw affecting the thigh, scrotum and penis.

FIG. 2. Craw-Craw affecting the left armpit.

FIG. 3. Craw-Craw of buttocks, three months' duration.

Fig. 1

Fig. 2

Fig. 3

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## EXPLANATION OF PLATE XVII

FIG. 1. Craw-Craw of buttock, one year's duration.

FIG. 2. Craw-Craw of wrist and hand, three years' duration.

FIG. 3. Craw-Craw of thighs, arms, and hands.

Fig. 1

Fig. 2

Fig. 3

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# THE MOSQUITOS OF ACCRA

BY

A. INGRAM

AND

J. W. S. MACFIE

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The following list of the mosquitos which have been collected at Accra, Gold Coast, West Africa, is of some interest as showing the great abundance of these insects in some places in Africa.

All the sixty-eight species were found within the municipal boundary of Accra. This area, which is about three and a half miles long and two miles broad, is situated on the sea coast and surrounded on the land side by an arid plain, mostly bare, uncultivated, grass-land. It includes the town of Accra itself, the suburbs of Christiansborg and Adabraka and their immediate environs, two lagoons, but no forest land. The area would not, therefore, appear to be exceptionally favourable for the maintenance of a large number of different mosquitos.

The area has been rather thoroughly searched for mosquitos during the last few years, by Graham (1908), by Connal (1912), and by ourselves (1914 to 1923), and, moreover, is now-a-days very carefully watched by the Public Health Authorities, to one of whom, Dr. P. S. Selwyn-Clarke, we are indebted for the larvae of several species not previously recorded. Nevertheless, it is probable that other species still await detection, for up to the present additions have been made almost every year, and some parts of the area have not yet been thoroughly explored.

Finally, it should be clearly stated that, notwithstanding the large number of species known to occur in it, Accra is not a place where mosquitos are exceptionally numerous or troublesome ; on the contrary, in the part where Europeans mostly reside they are comparatively rare, and the whole area must be regarded as usually relatively mosquito-free.

Mosquitos recorded as occurring within the municipal area of Accra :—

*Aedes* (*Aediomorphus*) *furcifer*, Edw.
*Aedes* (*Banksinella*) *fuscinervis*, Edw.
*Aedes* ,, *lineatopennis*, Lud.
*Aedes* (*Ochlerotatus*) *abnormalis*, Theo.
*Aedes* ,, *albocephalus*, Theo.
*Aedes* ,, *apicoannulatus*, Edw.
*Aedes* ,, *caliginosus*, Graham
*Aedes* ,, *domesticus*, Theo.
*Aedes* ,, *hirsutus*, Theo.
*Aedes* ,, *irritans*, Theo.
*Aedes* ,, *minutus*, Theo.
*Aedes* ,, *minutus* var. *biannulatus*, Theo.
*Aedes* ,, *nigricephalus*, Theo.
*Aedes* ,, *ochraceus*, Theo.
*Aedes* ,, *punctothoracis*, Theo.
*Aedes* ,, *tarsalis*, Newst.
*Aedes* ,, *wellmani*, Theo.
*Aedes* (*Stegomyia*) *apicoargenteus*, Theo.
*Aedes* ,, *argenteus*, Poiret (*Stegomyia fasciata*, F.)
*Aedes* ,, *luteocephalus*, Newst.
*Aedes* ,, *metallicus*, Edw.
*Aedes* ,, *simpsoni*, Theo.
*Aedes* ,, *unilineatus*, Theo.
*Aediomyia africana*, N-L.
*Anopheles* (*Anopheles*) *mauritianus*, Gpre.
*Anopheles* (*Myzomyia*) *costalis*, Lw.
*Anopheles* ,, *domicolus*, Edw.
*Anopheles* ,, *funestus*, Giles.
*Anopheles* ,, *marshalli*, Theo.
*Anopheles* ,, *pharoensis*, Theo.
*Anopheles* ,, *umbrosus*, Theo.
*Culex annulioris*, Theo.
*Culex consimilis*, Newst.
*Culex decens*, Theo.
*Culex decens* var. *invidiosus*, Theo.
*Culex duttoni*, Theo.
*Culex fatigans*, Wied.
*Culex grahami*, Theo.
*Culex guiarti*, Blanch.
*Culex horridus*, Edw. (*Protomelanoconion fusca*, Theo.)
*Culex* (*Micraedes*) *inconspicuosus*, Theo.
*Culex insignis*, Cart.
*Culex* (*Culiciomyia*) *macfiei*, Edw.
*Culex* ,, *nebulosus*, Theo.
*Culex ornatothoracis*, Theo.
*Culex perfidiosus*, Edw.
*Culex perfuscus*, Edw.
*Culex quasigelidus*, Theo.
*Culex rima*, Theo.
*Culex thalassius*, Theo.
*Culex thalassius* var. *fuscus*, Theo.
*Culex tritaeniorhynchus*, Giles.
*Eretmopodites quinquevittatus*, Theo.
*Ficalbia* (*Etorleptiomyia*) *mediolineata*, Theo.
*Lutzia tigripes*, Gpre.
*Lutzia tigripes* var. *fusca*, Theo.
*Mimomyia hispida*, Theo.
*Mimomyia plumosa*, Theo.
*Mimomyia splendens*, Theo.
*Mucidus mucidus*, Karsch.
*Mucidus scatophagoides*, Theo.
*Taeniorhynchus* (*Mansonioides*) *africanus*, Theo.
*Taeniorhynchus* (*Mansonioides*) *uniformis*, Theo.
*Uranotaenia balfouri*, Theo.
*Uranotaenia connali*, Edw.
*Uranotaenia mashonaensis*, Theo.
*Uranotaenia mayeri*, Edw.
*Chaoborus ceratopogones*, Theo.

# NOTE ON A POSSIBLE INTERMEDIATE HOST OF *SCHISTOSOMA HAEMATOBIUM* IN THE GOLD COAST

BY

A. INGRAM

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Attempts have been made on several occasions during the past eighteen months to determine the molluscan host or hosts of *Schistosoma haematobium* in the Gold Coast by exposing Bullinus-like snails, collected in pools and water-holes used as bathing places in the neighbourhood of Accra, to the attacks of miracidia hatched from ova obtained from the urine of cases of Bilharziasis in the colonial hospital. Three species of such snails are usually found in these bathing pools and they are, according to the identifications very kindly supplied by Colonel M. Conolly: *Isidora forskali*, Ehrn, *Physa waterloti*, Germain, and *Physopsis globosa*, Morelet. *I. forskali* is of much rarer occurrence than either of the others.

Dissections of specimens of these snails made as soon as they were brought to the laboratory, appeared to show that neither *I. forskali* nor *Physa waterloti* was naturally infected with furcocercous cercariae and no furcocercous cercariae were found in the livers of these species after they had been exposed to miracidia of *Schistosoma haematobium* and had been kept alive for a month to five weeks. On the other hand, dissection of newly-arrived specimens of *Physopsis globosa* showed an infection with furcocercous cercariae in 1·2 per cent. (2 in 165 examined), and 14 of 25 survivors of a lot of 76 of this species, exposed to miracidia of *S. haematobium* on the 9th July, were found to be infected with furcocercous cercariae when dissected on various dates between the 23rd August and the 16th September, 1923. It has, nevertheless, to be admitted that all attempts to infect rats with these cercariae proved unsuccessful; *e.g.* five *M. rattus* were placed in narrow glass cylinders and were kept for two hours semi-submerged in water to which had been added teased portions of livers of *Physopsis globosa* infected with furco-

cercous cercariae ; but although all the rats survived for more than six weeks after the experiment before dying or being killed, in no case were adult Schistosomata found in the liver or mesenteric veins. The dissection of three *M. decumanus* and of one *M. rattus* six to nine weeks after a similar exposure in water containing free-swimming cercariae, proved equally fruitless as regards the finding of Schistosomata in the livers or mesenteric veins, or the finding of ova in the bladders.

It may be noted that *Physopsis globosa*, Morelet, is the species of snail which Dye (quoted by Christopherson in a letter *Brit. Med. Jl.* of September 8, 1923, p. 437) in Nyasaland, observed to be attractive to the miracidia of *S. haematobium* and to be penetrated by them. It is, therefore, not unlikely that this species of snail can function as an intermediate host of *S. haematobium* in the Gold Coast.

# THE CROONIAN LECTURES ON LEPROSY RESEARCHES

BY

LT.-COLONEL SIR LEONARD ROGERS, C.I.E., M.D., F.R.C.P., F.R.C.S., F.R.S., I.M.S.

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## Lecture I. THE EPIDEMIOLOGY OF LEPROSY

When I had the great honour of being appointed to deliver the Croonian Lectures, I selected the subject of leprosy because I had spent my last few years in India in research on its treatment, and have since devoted several years to a study of its literature, while I believe it has not been discoursed on before this College since the late Dr. Robert Liveing delivered the Gulstonian Lectures in 1873, just when the epoch-making discovery of the lepra bacillus by Hansen was revolutionising our outlook on its etiology. Recent advances in treatment have at last furnished us with methods of dealing with the disease far more effectively than ever before, making the present time opportune for reviewing the whole subject. I propose in the first two lectures to deal with the epidemiology, mode of spread and prophylaxis of leprosy, and in the third to consider the improved methods of treatment.

### THE SPREAD OF LEPROSY OVER THE WORLD

Munro, in his learned articles in the *Edinburgh Medical Journal* of 1877-79, refers to an Egyptian record of 1350 B.C. of leprosy among negro slaves from the Sudan and Dafur, which is interesting in view of the present high leprosy rates in Central Africa, while very early records of India and China are believed to refer to leprosy. The first reliable records of its spread relate to the invasion of Europe through Greece about 350 B.C., probably by the armies of Darius, while those of Pompey carried it to Rome in 62 B.C. Galen mentions it in Germany in 180 B.C., and it spread all over Western Europe by the ninth century; both Sir James Y. Simpson and Sir George Newman

have recorded much interesting information regarding early leper hospitals in the British Isles, while Bergen had a leper hospital in 1266 and still has one to the present day.

Leprosy was, therefore, widely prevalent in Europe before the Crusades, but greatly increased from the eleventh to the thirteenth century, only to show a remarkable decline in the latter part of the fourteenth century. This, I believe, was partly owing to the Black Death of 1349, which is said to have carried off nearly half the population of Europe, for recent records show reductions of leprosy in Iceland and India due to epidemic disease and famines falling most heavily on outcast lepers; but also due, I feel sure, to the drastic segregation measures enforced in Western Europe during the Middle Ages; for, as Munro and others have pointed out, the disease only declined rapidly and eventually died out in just those countries where segregation measures were carried out, and lingers to this day in countries which neglected them, such as Scandinavia, the Iberian Peninsula, Russia, Turkey, and Greece.

While the disease was declining in Europe it was carried to the Western Hemisphere by the Spanish and Portuguese invaders, and later by the extensive negro slave trade from the very parts of Central Africa which still show the highest leprosy rates, and more recently still by Indian and Chinese immigrants. All the evidence goes to show that the aboriginal Indians were free from the disease, and those having little or no intercourse with leprous races from the old world still remain free to this day. As late as the middle of last century serious outbreaks of leprosy took place in several of the Pacific Islands, Hawaii having been infected by a Chinaman in 1848, according to Hildebrand, who saw the first indigenous cases five years later. The outbreak in New Caledonia was certainly due to a Chinese leper, who died about 1865 after living two years with a tribe, one of whom developed leprosy only a year later, and the disease spread so alarmingly that, according to Ortholon, within ten years from one-fourth to one-half the people in some places were attacked, while by 1910 no less than 90 per mille of the 8,000 convicts had become lepers. The epidemic soon spread to the neighbouring Loyalty Islands, the first case again being in a Chinaman affected in 1878, and by 1909 Nicholas found 35 per mille of the population to be lepers, although many cases were hidden. The

Marquesas Isles were next invaded, and by 1909 the leprosy rate had reached the appalling figure of 66·7 per mille (Buisson), or two hundred times that of India. Professor Jeanselme stated in 1903 that the Chinese had also 'carried leprosy to Indo-China, Siam, the Straits Settlements, Java, Sumatra, Borneo, the Philippines and other East Indian Islands, in several of which countries Chinese still form by far the majority of the infected,' Sir James Cantlie as early as 1897 having pointed out the rôle of the Chinese in spreading leprosy in these areas.

It is therefore clear that leprosy still retains its powers of spreading, and I fear there is good reason to believe that, with the opening up of communications and trade routes, the disease is now extending in tropical Africa, so that the problem remains a serious one for the British Empire. Thus the whole history of the spread of leprosy over the world is that of an insidious and slowly communicable disease carried by armies or the emigration of infected races to previously immune countries, leaving no doubt regarding the infectivity of the disease in some way or other, and of the necessity of safeguarding against its further extension.

## THE WORLD DISTRIBUTION OF LEPROSY AND CLIMATIC CONDITIONS

The close relationship between hot, humid climates and high leprosy rates, which I demonstrated last year by maps illustrating the leprosy rates per mille throughout the world and in India, respectively, is the most important epidemiological feature of the disease, as the following brief summary will show. Every area with the very high rates of 5 per mille and upwards (that is, fifteen or more times the present rate in India, where leprosy cases can be seen daily in the streets of every town) is situated within the tropics, having high annual rainfall, usually of sixty inches or more, *e.g.*, a large part of tropical Central Africa, with such extreme rates as 20 to 60 per mille on the Ivory Coast, the Cameroons and part of Eastern Belgian Congo, these constituting the most severely affected large area in the world ; while high rates are also met with in the hot damp countries of French and Dutch Guiana in South America, and in the Oceanic Islands, already mentioned as epidemically infected last century, and a few other areas.

In contrast with those high rates we have the remarkable fact that the only countries in the tropical zone, which have little or no leprosy, are the extremely dry areas of the Western Sahara, the French province of Mauretania, German South-west Africa, and the western coast of South America, all with less than ten inches of rain annually.

In the Northern Temperate Zone we also find the high leprosy rates of between 1 and 5 per mille in Norway and Iceland before active segregation measures were enforced, Japan and Korea, in all of which the rainfall is between 30 and 60 inches a year, against the usual rate for this zone of 10 to 30 inches.

Another important factor, however, comes into play in much of this zone, namely, the high degree of sanitary advance in Europe and North America greatly limiting the danger from returned emigrants infected with leprosy, with the result that the disease is no longer endemic in such countries.

The relationship between high humidity and relatively high leprosy rates and *vice versa* is illustrated in a most striking manner by my map of Indian leprosy distribution, rainfall and humidity data, the highest rates being met with in wet Burma, Assam, Bengal outside the deltaic region, Bihar, Orissa and the eastern Central Provinces, and the west coast of Bombay and Madras, which all have high monsoon rainfall, the leprosy rates in the Ganges valley actually decreasing with those of both the rainfall and the humidity in each division from east to west with the decreasing strength of the monsoon, while the humid Himalayan Kumaon Hills have ten times the leprosy rate of the hot, dry plains at their feet. On the contrary, the lowest leprosy rates are all met with in the dry North-western Punjab, North-west Frontier Province, Baluchistan and Sind, and in the dry central portion of the peninsula. The only exception is a somewhat high rate in comparatively dry Berar and the Deccan, probably due to infection by centuries of close trading with the people of the same race and language as those of the damp leprosy-infected western coast. Many other striking illustrations could be mentioned, such as higher leprosy rates in the rainy halves of Madagascar and Sumatra. I shall return later to the probable explanation of these facts.

## INVERSE RATIO BETWEEN LEPROSY PREVALENCE AND TUBERCULISATION

The remarkable relationship I found between damp heat and leprosy prevalence led me to collect data regarding tuberculosis in the tropics, and to study the writings on the subject of Colonel G. E. Bushnell and Professor Lyle Cummins. These showed that climate itself had much less influence on this disease than the degree of tuberculisation of the population in any country, as illustrated by a high percentage of positive von Pirquet reactions indicating the numbers infected with mild or latent tubercle in childhood, protecting them to a large extent from the more acute forms of the disease in later life. It is now well established that native races in Central Africa, Oceana and America, among whom some of the highest leprosy rates are found, have very low percentages of positive tuberculin reactions, and are liable to suffer from decimating epidemics of very acute generalised tuberculosis on becoming infected from chronic cases of phthisis in Europeans. Thanks to Professor Calmette, von Pirquet reactions have been worked out in most of the French tropical possessions, and on studying these and other data I found that a low degree of tuberculisation of the people occurred in just those areas where exceptionally high leprosy rates per mille have been recorded, as shown in Table I ; intermediate rates are seen in such long tubercular infected countries as India and China, while the highest percentages of von Pirquet reactions are met with in Western Europe, from the greater part of which leprosy died out as an endemic disease over a century ago, in a manner which is unknown in any other part of the world.

In this connection it may be recalled that tuberculin gives reactions in leprosy, that such an acute observer as the late Sir Jonathan Hutchinson suspected that the tubercle bacillus may be modified by some element in badly cured fish to assume the power of producing leprosy, while such an eminent pathologist as the late Professor Sheridan in 1891 reported a case of leprosy with lung lesions in which he suggested the possibility ' that leprous lesions are the result of a *modified form of tuberculosis which tends to return to the normal type in the lungs.*' The italics are in the original. Although I do not for a moment endorse these views, they at least emphasise the close relationship of the two diseases, and suggest

as the most likely explanation of the interesting data in the table the possibility that infection by mild tubercle of a large proportion of a population may render it less susceptible to the infection of leprosy, this being one factor in the extinction of leprosy as an endemic disease in Western Europe, in addition to improved hygiene and other influences.

TABLE I

Leprosy Rates per Mille and von Pirquet Reaction Percentages.

| Country | Leprosy per Mille | Von Pirquet Percentages |
|---|---|---|
| France (Lille) ... ... ... ... ... | 0·0 | 87·7 |
| Austria ... ... ... ... ... ... | 0·0 | 95·0 |
| United States ... ... ... ... ... | Extremely low | 94·9 |
| Indo-China ... ... ... ... ... ... | 0·67 | 48·0 |
| Martinique ... ... ... ... ... ... | 1·16 | 57·0 |
| India ... ... ... ... ... ... ... | 0·32 | Fairly high |
| French Guinea ... ... ... ... ... | 5·0 | 12·0 |
| Senegambia ... ... ... ... ... ... | 10·0 | 15·2 |
| Tanganyika ... ... ... ... ... ... | 7·0 | 21·0 |
| Cameroons ... ... ... ... ... ... | 20·3 | 4·4 |
| Ivory Coast ... ... ... ... ... ... | 60·7 | 8·4 |

However this may be, the reverse is certainly not the case, as it is well known that the most frequent fatal complication of advanced leprosy is pulmonary tuberculosis, and one which greatly complicates treatment, for doses of chaulmoogra oil derivatives which are of value in leprosy are actively harmful to the tubercular complication, although sodium morrhuate in small doses may sometimes be used with advantage in such cases.

## NUMBER OF LEPERS IN THE WORLD AND IN THE BRITISH EMPIRE

Some ten years ago Dr. Victor G. Heiser estimated the world's lepers at 2,000,000, since when further data have come to light, especially regarding the great prevalence in Central Africa, and a close study has convinced me that 3,000,000 would be a more correct figure, including the following recorded data :—

In EUROPE 7,000, with about 1,000 each in South Russia, the Baltic Provinces and Crete, and about 500 each in Turkey, Roumania, Spain, and Portugal.

In ASIA 1,250,000, including 500,000 to 1,000,000 in China with its dense population, 102,000 in both Japan and India, 15,000 in both Indo-China and Siam, 5,000 in both the Philippines and in the East Indies, and smaller numbers in Malaya, Ceylon and Palestine, etc.

In AFRICA 500,000, nearly all in tropical Central Africa, although the estimate is only a very approximate one, the data being nearly all based on the percentages found in the examination of a few thousand persons in different areas. Other recorded data are Egypt 6,500, Madagascar 4,000, and South Africa 2,500.

In the WESTERN HEMISPHERE 30,000, including Brazil 15,000, Guiana 3,000, Cuba 1,500, the British West Indies 1,000, Colombia 6,500, Venezuela 750, etc., while Oceana has about 5,000.

All the above figures relate only to typical advanced cases, while considerable infected areas remain for which no data are available, and it is safe to say that there is at least one early unrecognised case for every recorded typical one, Muir's Indian experience leading him to think that even this is an underestimate. Yet the recorded figures total 1,792,000, so that, including early unrecognised cases, 3,000,000 is far from being an improbable number for the whole world, constituting leprosy a problem of unsurpassed difficulty and importance when the terrible nature of the disease is borne in mind.

Turning next to the number of lepers in British territories, I have entered the main data I have been able to collect from an examination of recent Colonial medical reports and other records in Table II, and although not complete, the total number comes in round figures to 156,000, two-thirds in India, and the greater part of the remaining

third in Africa, so that when allowance is made for an equal number of early unreported cases, there must be at least 300,000 lepers in our empire, the control of which is not the least of the white man's burden.

The important question, whether leprosy is increasing or decreasing, can best be studied by an examination of the following census returns of the number of lepers in British India during the last six decades, those of the British governed territories being selected, as this area, except for the small inclusion of Upper Burma, has remained unchanged since 1881.

| | | | | | | |
|---|---|---|---|---|---|---|
| Census ... | 1872 | 1881 | 1891 | 1901 | 1911 | 1921 |
| Lepers ... | 101,590 | 118,953 | 110,509 | 85,878 | 92,433 | 85,122 |
| Per Mille ... | 0·55 | 0·60 | 0·56 | 0·37 | 0·38 | 0·34 |

Table II

Leprosy Incidence in the British Empire

| Area | Year | Lepers | Per Mille | Remarks |
|---|---|---|---|---|
| India ... ... ... | 1921 census | 102,513 | 0·32 | |
| Ceylon ... ... ... | 1921 | 577 | 0·13 | |
| Malay States ... ... | 1921 | 450 | 0·34 | In leper institutions |
| British N. Borneo ... | 1919 | 54 | ... | In leper institutions |
| Fiji ... ... ... | 1920 | 450 | ... | In leper institutions |
| West Indies ... ... | 1921 census | 1,189 | 0·74 | |
| British Guiana... ... | 1921 ,, | 247 | 0·83 | |
| Cyprus ... ... ... | 1921 | 74 | 0·23 | In leper institutions |
| Africa :— | | | | |
| Nigeria ... ... | 1921 census | 32,000 | 3·2 | |
| Tanganyika ... ... | 1921 | 7,026 | 0·7 | 1 in every 589 of population |
| Kenya ... ... | 1922 | 2,018 | 0·74 | 6 in 8,067 persons examined |
| Uganda ... ... | ... | ? 3,000 | 1·0 | 303 lepers died in 1919 |
| Nyasaland ... ... | 1921 census | 1,666 | 1·39 | Reported to be spreading |
| S. Rhodesia ... ... | 1921 | 1,000 | 1·11 | Estimated at least 1,000 |
| S. Africa ... ... | 1923 | 2,501 | 0·46 | In leper institutions |
| Mauritius ... ... | ... | 600 | 1·6 | |
| Palestine ... ... | 1902 | 600 | 0·86 | Jeanselme |

Unfortunately, the striking decline in the number and the rate per mille revealed in the figures of 1901 was almost entirely due to the issue of special directions excluding cases of leucoderma and syphilis, incorrectly returned as leprosy in the first three censuses, and so is not a real decline. A small part of the decline between 1891 and 1901 was due to a series of famine years, being followed by a slight increase in 1911, while the slight fall in 1921 was probably due to the influenza epidemic of 1918-19; so we may conclude that leprosy has been fairly stationary with a slight tendency to decrease during the last sixty years, during which prophylactic measures have been practically negligible. The scare that was raised about 1890 that leprosy was increasing rapidly in India, and which led to the appointment of the Indian Leprosy Commission in 1891, is, therefore, baseless as that Commission concluded, and there are good reasons for hoping that the more effective measures against the disease now available, if only they can be fully utilised, will lead to a decline of leprosy in India in the next few decades. Although the census figures have a relative value they only include the advanced, easily recognised cases, and Dr. Muir thinks the real numbers may be as high as between 500,000 and 1,000,000 if all the early cases are included, so it may be well to point out that the first result of the more general adoption of improved methods of treatment will be an apparent increase of leprosy, due to the early and more amenable cases coming forward from their hiding places and declaring their disease, instead of hiding it as long as possible. This is already evident in the Philippines, Hawaii and elsewhere, and so far from being a real increase, it will be the first promising sign of the possibility of controlling and eventually reducing the incidence of leprosy.

The AFRICAN problem is a still more difficult one, as the real numbers in our extensive territories are quite unknown, while the poverty and the small medical staffs compared with the population and extent of the countries greatly enhance the difficulty of dealing effectively with the disease. On the other hand, the smaller number of lepers in the West Indies, Malay States, Fiji and other smaller colonies afford more hopeful fields for continued efforts to reduce the disease by the methods to be described in the next lecture, when the prophylactic measures already in force in several of our colonies will also be dealt with.

## GENERAL CONDITIONS INFLUENCING THE PREVALENCE OF LEPROSY

Having now formed some idea of the prevalence of the disease throughout the world and in our empire, I next turn to a consideration of certain general conditions which materially influence the incidence and spread of leprosy, and require to be taken into account in devising prophylactic measures in different countries.

*Stage of Civilization and Hygiene.* One of the most potent general factors is the hygienic condition an infected race has reached ; this is well illustrated by the contrast between the conditions prevailing in Europe during the great prevalence of leprosy in the middle ages, as compared with the present time, when leprosy-infected Europeans returning from the tropics very rarely infect others under the advanced hygienic conditions in Western Europe within the temperate zone. The sanitary conditions under which the majority of the population of Great Britain dwelt during the eleventh to the fourteenth centuries cannot have been very far removed from those of the vast populations now living in one-roomed huts in India, China, Oceana and central Africa, where leprosy is now most prevalent. That such conditions are more potent than racial susceptibility to the disease is well shown by the reduction in the prevalence of leprosy, by improvement in their housing accommodation, among the large number of Scandinavian immigrants to Minnesota and neighbouring north-central states of America in the latter part of last century. Hansen himself visited the United States to trace the progress of leprosy among 170 Scandinavians, who had crossed the Atlantic from Norway when they were either already suffering from leprosy or developed the disease within the possible long incubation period after leaving their native country ; and he failed to find a single new infection in two generations, although leprosy continued to be prevalent among the same class in Norway itself. In explanation of this most interesting fact he pointed out that, whereas in their own country they had lived in such small and overcrowded houses that it was customary for all the males to sleep in one room and the females in another (as late as 1891, 42·4 per cent. of Norwegian town dwellings, according to Newsholme, having only one room), in Minnesota the immigrants built large houses, each man having his own bedroom, or at least his own bed,

thus greatly limiting the overcrowding which is such an important factor in furnishing opportunities for infection. The cool climate was also, doubtless, an important factor in addition to improved hygiene, for in the hot, humid, southern state of Louisiana, leprosy spread during the same period of last century among well-to-do French families, once more illustrating the influence of unfavourable climatic conditions. The effect of overcrowded one-roomed huts in favouring the spread of leprosy has also been pointed out by Green in Hawaii and Hearsay in Nyasaland.

Promiscuity both general and sexual, greatly favoured by overcrowding, is another most important factor in the spread of the disease, Buisson stating that the Marquesas Island epidemic was favoured by horrible sexual promiscuity ; the reports from Hawaii, where the women seldom knew who were the fathers of their children, laid great stress on the importance of the same factor, while more than one writer recorded that every European infected with leprosy was known to have had intimate relations with the native women ; Hillis reported the same occurrence in British Guiana and Drognant-Landré in Surinam and Jamaica ; Sir George Newman mentions the unrestrained sexual relationships in the middle ages in our own country as having favoured the increase of leprosy. Even in recent times in the province of Galicia in Spain, the most unusual feature of a high leprosy rate among females was attributed by Tello to their promiscuous habits during the temporary emigration of their husbands.

Other favouring social conditions, to which the spread of leprosy has been attributed in Oceana and other tropical countries with a low degree of civilization, include eating out of the same dish and smoking a common pipe, while Hansen recorded that in Norway, during the frequent visits of the socially inclined people to relatives and friends, it was considered very bad form to object to sleeping in the same bed as a leper, unless he was in a very advanced stage of the disease.

Absence of all fear of leprosy favoured the spread of leprosy in Oceana and among the fatalistically inclined Mohamedans of Central Africa, as well as in Norway seventy years ago, before segregation measures had educated the people to realise the danger ; Munch pointed out the same thing in South Russia. On the contrary, various cruel customs among savage races have been recorded as

having reduced the incidence of leprosy in Senegal, the Ivory Coast, Nigeria, Madagascar before the arrival of the French, Nyasaland, Zululand, Sumatra, India and other countries.

Spread of leprosy by immigrants has already been mentioned in tracing the infection of America and Oceana, to which may be added the following instructive modern example in British territory. In 1863, for political reasons, leprosy-infected Hottentot tribes of West Griqualand were moved right across the Orange Free State to Griqualand East to the south of Natal, with the result that they infected both the country they passed through and their new head-quarters at Kokstad, and the disease continued to spread among them until in 1895, 558 cases were known ; the neighbouring Basutos also became infected, but partly through returned miners.

The escape of tribes having no intercourse with leprosy-infected neighbours is illustrated by the absence of leprosy to this day among the more remote American-Indian tribes of Brazil, Guiana and other South American countries ; this is clearly not due to any lack of susceptibility to the disease, for Hillis recorded long ago that in British Guiana the Warara tribe, which alone had close relations with the infected negro population, including intimate relationships with some of the female inmates of the leper asylum in its early days when the administration was defective, did contract leprosy.

All the above epidemiological features of leprosy point to the disease being in some way or other a communicable one ; during the latter half of the nineteenth century a great controversy raged on the subject, in which a Committee of this College played an important, but it is now clear, an unfortunate part, due to lack of knowledge in those pre-bacteriological days. As, in spite of the lepra bacillus having been discovered just over fifty years ago, the precise manner in which the organism passes from the diseased to infect the healthy is still not completely settled, many of the facts brought forward during those controversies are still of value in the elucidation of the problem. I believe, however, that no leprologist of repute any longer doubts that the disease is a communicable one, this having been universally admitted by all recent leprosy conferences, including the third international one at Strasbourg last year.

## CONTROVERSIES ON THE HEREDITARY AND CONTAGIONIST THEORIES OF LEPROSY CAUSATION

From the time of Moses right down through the middle ages, exaggerated views were held regarding the contagiousness of leprosy, doubtless due to the confusion with it of other more highly infectious skin diseases, and they resulted in the cruel segregation laws of those times. About the middle of the seventeenth century, when leprosy had nearly disappeared as an indigenous disease in Western Europe, the pendulum swung to the opposite extreme, and contagion was altogether denied ; the spread of the disease was attributed solely to heredity, just as was the case with the closely allied tuberculosis, while leprosy was also described as a ' blood dyscrasia,' and these views were still nearly universally held down to the time of Hansen's discovery of the lepra bacillus in 1873, and even after that event were maintained by some authorities, although the infective theory steadily gained ground from that moment. The most influential supporters of the hereditary theory were the great Norwegian authorities, Danielssen and Boeck, who wrote the first accurate description of the nodular and anaesthetic types of leprosy in their book of 1848, in which they supported the hereditary theory with figures relating to 213 cases ; these were for long accepted as proving their point, although when examined in the light of more recently discovered laws of heredity they have precisely the opposite bearing. The Norwegian authors regarded as proof of heredity the occurrence of two cases of leprosy within four generations of a family, even if the first case was in a grandchild and the second in a grandparent ; this method of calculation showed direct descent in 32·4 per cent. and indirect in 54·5 per cent., the remaining 10·1 per cent. being attributed to spontaneous origin, the possibility of infection in a household or family being completely ignored, while their table showed more cases in the second and fourth than in the first and third generations, as well as more in the collateral than in the direct line of descent. These data are, therefore, in reality valueless as evidence of leprosy being essentially an hereditary disease. Later Norwegian authorities, including Hansen, opposed this theory, Holmsen, for example, finding only 12 out of 93 lepers whose parents or grandparents had suffered from the disease, and

no less than 11 of the 12 had been born before their parents or grandparents were attacked, while Munro, Vandyke Carter, J. C. White of the United States, Ehlers and other authorities might be quoted to the same effect.

Still more conclusive positive evidence against the hereditary view is afforded by the children of lepers being too few to allow of leprosy surviving more than two or three generations if it were solely hereditary ; this is shown by Munro, by Lewis and Cunningham, who found only five surviving children of 52 lepers in India, and by Arning in Hawaii, where the disease spread far too rapidly to be hereditary ; by the absence of hereditary transmission among the Scandinavian lepers in Minnesota and among many hundred children of lepers brought up by missions in India and elsewhere after early separation from their leper parents ; and by the infection of numerous Europeans in the tropics, whose ancestors had been free from leprosy for many generations. Virchow thought that only a predisposition to the disease was inherited, as was so long held with regard to tuberculosis, but I have found no evidence in recent medical literature in favour of that view. Nor is the question of purely academical importance, for the anticontagionist school argued that segregation of lepers must be useless because the disease is solely hereditary in origin.

*Hutchinson's Fish Theory.* The late Sir Jonathan Hutchinson's fish theory may conveniently be dealt with here, as it is also of pre-bacteriological origin, dating from 1863, in its first form. It held leprosy to be ' fish-eater's gout ' dependent on excessive consumption of badly preserved fish, but modified after the discovery of the lepra bacillus to mean that a particle of badly cured fish might produce leprosy developing many years later : a difficult event to disprove, although its author later admitted that his theory would not explain the spread of leprosy among non-fish eating Basutos. This fact led him to admit in addition ' commensal communication ' through eating food other than fish contaminated by a leper ; I do not know of any surviving supporter of the theory.

## THE COMMUNICABILITY OF LEPROSY

*Historical.* The history of the gradual re-establishment of the infectious theory of leprosy is an interesting one. In 1862, at the request of the Colonial Secretary, this College appointed a Committee to report on the infectiveness of leprosy. This Committee issued a bulky volume of evidence collected by means of a questionnaire from the Colonies and India, and concluded that leprosy was 'not contagious or communicable to healthy persons by proximity or contact with the disease,' but 'is essentially a constitutional disease indicative of a cachexia or depraved condition of the general system,' and that there was no evidence that 'would justify any measures for the compulsory segregation of lepers.' On the strength of this report the Colonial Secretary issued orders to repeal all laws in our possessions affecting the liberty of lepers (as the direct result of which, increases of leprosy have been recorded by Hillis in British Guiana, Munro in St. Kitts, Broes van Dort in the East Indies and Drognant-Landré in Surinam), although, if Vandyke Carter's statement is correct, none of the College Committee had any material experience of leprosy, and Dr. N. C. MacNamara, a distinguished Indian Medical Service Officer, came to diametrically the opposite conclusion from a careful analysis of the evidence from India published by the Committee. This unfortunate report had one good effect, namely that it stimulated leprologists with experience in leprous tropical countries, such as Drognant-Landré in 1869, Munro 1877, Brousse 1879, Hillis 1881, etc., to record convincing evidence of the communicability of leprosy. The last discordant note was sounded by the Indian Leprosy Committee of 1892, which appears to have been dominated by the ablest anti-contagionist leprologist of the day, Dr. Bevan Rake, nominated by this College, who reported that leprosy arose *de novo*, a view which was repudiated by the London Committee of the National Leprosy Fund who sent out the commission.

*Evidence of the Communicability of Leprosy.* The greatest difficulty in demonstrating the communicability of leprosy lies in the long incubation period, averaging several years, of the disease, which in many cases makes it impossible to trace the source of infection in endemic areas, aided by the insidious onset of the symptoms in

many cases and the tendency of patients to hide their disease. The following examples of the spread of the disease in newly infected areas, however, leave no room for doubt. In Louisiana a French woman developed leprosy in 1866, and within the next eleven years three of her four sons, one of two daughters, a nephew, an unrelated girl who nursed the mother, and an unrelated young man, who had frequently slept with the fourth infected son, all developed leprosy, although there were no other known cases in the country at the time ; the disease subsequently spread in the neighbourhood and remains to the present time. About the same time a French woman of Cape Breton Island of Western Canada, who was born opposite the Tracardie Leper Asylum of New Brunswick, developed leprosy at the age of 52, and within a few years five of her children, two of her grandchildren, a son-in-law, a man who attended the fourth infected son, and another man who was accustomed to sleep with the fourth infected son, all contracted leprosy when there were no other cases on the island ; all but the last case had died by 1881.

The carefully recorded Memel outbreak in East Prussia is equally instructive, for between 1848 and 1880 five Russian leper servant girls came to work in German families in this previously uninfected district, and by 1908 no less than 77 cases of leprosy had been traced directly or indirectly to these five original foci of infection ; the disease was still on the increase in 1899, infections nearly always occurring after long contact with a leper living in the same house. For example, four years after one of the Russian girls came to reside in a house, the father and his three children became lepers, and in a neighbouring friendly family the mother, three children, a female servant and the housewife's second husband were all attacked, and this second family infected the father, one son, two daughters, a daughter-in-law, a maid servant and two men servants of a third family, no less than 18 cases being thus traced to this one Russian leper girl.

*Frequency with which Infections can be traced.* In spite of all the difficulties in tracing the sources of infection of leprosy, Denny in the Philippines obtained histories of previous contact with leper relatives in 29 per cent., and McCoy in Hawaii and Gregory in Cape Colony in 37 per cent., although compulsory segregation laws made the patients loath to acknowledge infected relatives, while the percentages who acknowledged contact with lepers either relatives

or others were 60 per cent. in Dehio's South Russia cases and in Boyd and Warren's Texas series, 79·6 per cent. in Long's Basutoland, and 89 per cent. in Kereval's Caucasus cases; remarkably high figures under the circumstances, especially when it is remembered that unrecognised cases in an early stage of leprosy may sometimes be infective.

The most frequent sources and conditions of infection remain to be considered to complete this part of my subject. In this connection the relatives from whom infection is most frequently contracted may first be dealt with, the most significant facts being that in both the Philippines and Hawaii 84 to 85 per cent. of infections among relatives were those of children from parents or grandparents or from uncles or aunts, among brothers and sisters and between cousins, nearly all of whom are likely to be children and adolescents not over twenty years of age, while only 6 to 14 per cent. were older generations infected from younger ones, and the remaining 2 to 9 per cent. were conjugal infections: figures which confirm in a striking manner the evidence I shall adduce later to show that young persons are much more susceptible to leprosy than older people. Roumanian figures of infections from relatives show the same incidence, and also bring out the fact that in addition to 60 per cent. of infections of relatives, 5·5 per cent. were house infections of others such as servants, and the remaining 30 per cent. were due to other close associations.

The proportion of healthy persons living with lepers who become infected has also been investigated with very close agreement among the investigators shown by the fact that the data recorded by McCoy in Hawaii, Gregory in Cape Colony and by the Indian Leprosy Commission are respectively 4·2, 4·5, and 5·5 per cent. while in Japan and in Norway the figure in both cases was 2·7 per cent. The percentage of infections among conjugal couples are very similar, varying from 2·6 in Cape Colony, 3·8 in Japan and Norway, to 5 in Basutoland, 5·1 for males and 4·8 for females in Hawaii and 6·5 per cent. in India. Thus in both cases only about one in twenty of persons living in close association with a leper contract the disease, showing that leprosy is far from being a highly contagious disease; in fact probably not more so than tuberculosis itself under very similar conditions.

Further light on the subject is shown by a study I have made of

700 cases, in which the probable source of infection was traced and recorded in the literature of the last sixty or seventy years; an analysis of these I have already published and it may be summarised here :—

In 12·14 conjugal infection, and in 6·14 cohabitation, or 18·28 per cent. In 25·7 house infection, in 5·0 room and in 9·14 bed infection, or 39·84 per cent. Infection due to attending on a leper occurred in 19·87 per cent. Close association in 16·14, and from a leper playmate in 3·28 or 19·42 per cent. From wetnurse 1·14 per cent., wearing a leper's clothes 0·43 per cent., vaccination 0·59 per cent. and inoculation from a leper in 0·43 per cent. completes the total.

In view of the old anti-contagionist arguments that infection rarely resulted from conjugal relationship or from attending on lepers, it is interesting to note that almost one-fifth of my collected cases fell under each of these headings. Further, if the bed infections are added to those due to conjugal or cohabiting relationship nearly 30 per cent. of the infections occurred after sleeping in the same bed with a leper, while as most of the cases of attending lepers were among those nursing private cases in the patient's house, if we add a little over half of those cases due to attendance to the conjugal and house infections, we find that at least 70 per cent. of the infections took place as the result of living in the same house with a leper, usually for a considerable time: once more emphasizing the fact that long and close contact with a leper commonly precedes infection and that it is essentially a house infection, yet again bringing out a closely parallel condition with tuberculosis.

Although long and close association usually precedes infection, very short contact may suffice, three cases being on record in which young Europeans in the tropics developed leprosy within ten months to two years after cohabiting with a leper woman on a single occasion, at least one of them being under the influence of alcohol at the time. It is interesting to note that in Nigeria, Madagascar and China, native opinion firmly believes in leprosy being contracted by sexual intercourse, while lepra bacilli have been found in the semen, in lesions of the penis and vulva in lepers; the greater frequency of leprosy among males and at the age of adolescence might be explained by infection during such close relationships, although not necessarily directly as in venereal disease, as the frequency of

the discharge of innumerable lepra bacilli from the nose may be an important source of infection.

Numerous further examples of house infections might be given, the danger from leper servants for example, as shown by the Memel cases, and confirmed by Landré's 12 cases of pure European children being infected from leper servants. In well managed leper asylums the danger to attendants is almost negligible, but in the early days of the Molokai settlement of Hawaii with very defective adminstration, no less than 16·4 per cent. of infections among 244 healthy attendants were detected within three years, chiefly contracted through intimacy with the leper females. Doctors have occasionally become infected, sometimes through gross carelessness and neglect of ordinary cleanliness. Although in so many cases the probable source of infection can be traced, yet occasionally a European living under the most favourable hygienic conditions is mysteriously infected, possibly through clothes infected during washing or other unsuspected or indirect contact with a leper, such inexplicable cases in Europeans under very favourable hygienic conditions in India having come within my knowledge, so that no one can be considered quite safe while living in a leprous endemic area, however slight the risk may be.

The greater infectivity of the nodular type of leprosy is another important factor which is still too little recognised in prophylaxis, although well known to experienced leprologists. It is due to the copious discharge of lepra bacilli from ulcerated nodules and from nasal lesions in about 80 per cent., while in the anaesthetic form the bacilli cannot escape from the nerve trunks and are only discharged in the nasal mucus of 6 to 15 per cent. ; in advanced chronic nerve cases the bacillary infection often dies out with loss of all infectivity, but not until permanent crippling has resulted. These cases sometimes constitute 75 per cent. of leper asylum cases in India and elsewhere, their isolation being of little prophylactic value. This difference in infectivity is well brought out by the analysis of my 700 collected cases, for in 113 of them the type of the infective case was recorded, and in no less than 94·7 per cent. they were nodular, and only the remaining 5·3 per cent., or one-twentieth, were of the anaesthetic type. In Norway special care was rightly taken to isolate as many as possible of the infective nodular cases,

while only last year the 2,501 lepers in the South African asylums were examined from this point of view and no less than 693, or 28 per cent., were released as uninfective, thus providing valuable accommodation for infective cases.

*Age susceptibility of leprosy* is an equally important point, which may be illustrated by the figures of over 4,000 cases in which the age at the probable date of infection, or on the first appearance of symptoms (from which the average incubation period of three to five years must be deducted), was recorded by different observers. An analysis shows that infection took place within the first 20 years of life in 50 per cent., by 25 years of age in 66 and by 30 years of age in 75 per cent., after which the susceptibility is greatly decreased.

The great susceptibility of children to leprosy is well brought out by Denny's Culion figures and the 1922 data from the same place, showing that no less than 44 per cent. and 33 per cent. respectively of the children of leper parents living with and brought up by them became infected with leprosy ; Mouritz reported 34·6 per cent. of similar infections in Hawaii, while Drs. Lie and Sand of Norway recorded that among 2,010 children of 587 couples, 7 per cent. became infected when the father alone was a leper, 14 per cent. from the mother alone, and 26 per cent. when both parents were lepers, the infection of children exposed to infection thus being five to nine times as frequent as among conjugal couples. As all the evidence goes to show that leprosy is extremely rarely, if ever, congenital, the importance of separating healthy children from their leper parents from birth cannot be exaggerated ; a case has recently been reported in which a child removed from leper parents two months after birth developed the disease three years later, although, fortunately, such early infections are very rare.

I have now completed my survey of the distribution, epidemiology and of the various factors influencing the spread and infection of leprosy, and cleared the ground for considering the mode of infection and prophylaxis of the disease in my next lecture.

## Lecture II. MODE OF INFECTION AND PROPHYLAXIS OF LEPROSY

Fifty years ago Hansen discovered the lepra bacillus, which is now generally accepted as the cause of the disease. The precise manner in which the organism gains access to the human system is still unproved, owing to the failure to discover reliable methods of cultivating it or of infecting animals with the disease, and the equivocal results of attempts to infect man himself, Arning's positive result in Hawaii of inoculation of a convict having been invalidated by opportunities this man had of being infected from leprous relatives. In the case of rat leprosy, due to another acid-fast bacillus, however, Professor Marchoux has shown that the disease is inoculable, and it may be said at once that there is a remarkable consensus of opinion among experienced leprologists that human leprosy is also conveyed by inoculation of the organism through the skin or the nasal mucous membrane ; but as proof is wanting, it is necessary to consider the indirect evidence bearing on the probable mode of infection of the disease, and it was for this reason that I dealt so fully with the epidemiology of leprosy in the first lecture.

### POSSIBLE MODES OF INFECTION

Inhalation has for long been regarded as the common mode of infection of pulmonary tuberculosis, although the work of Calmette has thrown grave doubt on its frequency. In leprosy the lung is very rarely affected and never primarily as far as I know, while Muir has found that the lung lesions respond to treatment more rapidly than the skin ones. There is thus no evidence that inhalation is a common mode of infection in leprosy.

Infection through the ingestion of food was thought to be possible by Hutchinson, but I can find no valid support for this view in medical literature, although it is difficult to exclude the possibility of the lepra bacillus passing through the gastro-intestinal mucous membrane and lymphatic glands to reach the blood stream without leaving any visible trace of its passage, however unlikely this hypothesis may be. Moreover, the incidence of leprosy in households rarely shows several members developing the disease at about the same

time, as in food infections, the cases being nearly always separated by a year or more, as if due to separate accidental infections by another route.

Inoculation through the skin, on the other hand, has been thought to be the common mode of infection in leprosy by such authorities as Vandyke Carter as early as 1867, Hansen and Looft, Kaposi, Munro, White, Besnier, Hillis, Mouritz in Hawaii, Tonkin of Africa and many others. The only difficulty in marshalling the evidence in favour of this view is to select from the innumerable records bearing on it, of which the following are some of the most striking.

Hillebrand reported the infection of a European child in Borneo, who thrust a thorn into his flesh immediately after a native leper boy had inserted it into his own flesh ; and Solano met with a similar case in Colombia, in which a boy aged six thrust needles into his flesh immediately after a negro leper playmate aged eight had introduced them into nodules on his limbs, with the result that the first boy soon after began to get febrile attacks and pains in his limbs, and one year later was covered with a typical tubercular leprotic eruption. These two cases are of especial significance in view of the subjects being children of a highly susceptible age, whereas all the negative experimental inoculations I have found recorded were in far less susceptible adults, none of those inoculated by Profeta having been under 25, while the majority were over 30 years of age, when susceptibility is small.

Even more conclusive are the following two cases of doctors inoculated while operating on lepers. Dr. Ehlers reported the case of a Danish doctor in the West Indies, who wounded a finger during an obstetrical operation on a leper negress ; the wound healed slowly and he soon after developed in the injured finger severe pains, which later proved to be due to anaesthetic leprosy ; in Hundadze's case a medical man inoculated a wound of his right finger in opening a leper's abscess ; the wound healed normally, but two months after the site of the wound became red and swollen, this symptom being accompanied by fever for three days, and three weeks later red leprous patches appeared on the injured finger ; these were at first taken to be syphilis, but proved to be leprosy, leaving no doubt of infection through the wound. Hatch in Bombay has recorded a case of anaesthetic leprosy of the ulnar nerve closely

following a wound made while doing a post mortem on a typical leper, the diagnosis being confirmed by Vandyke Carter.

These cases, among others which might be quoted, should suffice to establish the possibility of leprosy being inoculated through the skin, while the close resemblance between limited dermal leprosy and tubercular dermatitis, now generally accepted as due to inoculation with the tubercle bacillus, presents an interesting analogy in a closely allied organism.

Vaccination as a possible cause of leprosy has given rise to much controversy and some loose statements, but very suggestive facts are on record; the possibility of its occurrence receives strong support from Arning's observations in Hawaii, proving that lepra bacilli were frequently present in vaccine lymph taken from apparently healthy skin in persons with extensive cutaneous leprosy, but not in vaccine from purely nerve cases, and he considered that there was strong evidence that leprosy had been spread in certain parts of Hawaii by carelessly performed arm to arm vaccination. Cases highly suggestive of infection in this manner have been recorded by Hillis, Gairdner of Glasgow and others, but as they occurred in endemic areas other modes of infection cannot be excluded; it is, however, of interest to note that Gavin Milroy records that Creole families of the West Indies obtained their lymph from the United States for fear of leprosy. Quite recently new light has been thrown on the subject by the observations of O. E. Denny and R. Hopkins at the United States Leprosarium, where after vaccination of both the healthy staff and the lepers, the latter alone had severe reactions; in eleven of them local swelling, fever and the appearance of fresh nodules and even ulnar neuritis appeared; so that it is possible that such reactions in early unrecognised or latent cases of leprosy may formerly, in Hawaii and elsewhere, have been taken for infections due to vaccination. In any case, infections clearly due to vaccination in leprous countries by the old arm to arm method can have very rarely resulted, while they are impossible with the general use of calf lymph at the present time.

Infection from clothes and bedding has frequently been suspected, Tonkin regarding this as a common occurrence in badly infected Central Africa from Nigeria to the Sudan, where it is the custom for clothes to be passed down, without ever being washed, from the

rich to the poorest until worn out ; to the sleeping of whole families together on mats in Hawaii, India and elsewhere have been attributed inoculations over the buttocks, shoulder-blades and cheeks ; a case of a primary lesion in the last position in a child, who slept on the bed of a leper relation, having recently been reported by Muir in Calcutta ; wearing lepers' clothes has also been recorded as a cause of infection in the West Indies and Norway, etc., while in Riga a large proportion of the few lepers occurred among washerwomen : all indicating possible infection through inoculation from clothes or bedding soiled with leprous discharges containing enormous numbers of bacilli.

Sites of primary lesions as evidence of inoculation afford further important evidence, the especial frequency of anaesthetic leprosy in bare-footed races having been recorded in Abyssinia, where ten out of twelve lepers are said to be of this type, the Sudan, Hawaii, Crete, Java (with 50 per cent. of such cases according to Gell), Peru and India. Only last year Muir published diagrams of the distribution on the surface of the body of the first noticed lesion (most of which are considered to be the probable primary sites of inoculation), of no less than 1,056 lepers in Indian asylums and noted that they greatly predominated on exposed parts, such as the face, and extensor surfaces of the extremities, as well as over the buttocks and shoulder-blades exposed to friction on bedding and sleeping mats. Further, in two areas on alluvial soil, primary lesions on the feet were very uncommon, but in two separate hilly and stony districts among the bare-footed they were frequent on the feet ; all facts indicating inoculation through the skin as the most usual mode of infection.

Inoculation through the nasal mucous membrane is another possible mode of infection first pointed out by Professor Jeanselme in 1897 and supported by Stricker, Muir and others, but recent examinations of a number of children of lepers in the Philippines for early signs of the disease showed none in which the nasal mucous membrane was infected without skin lesions, although the opposite occurrence was common, so that primary nasal infection is probably much less frequent than that through the skin.

The rôle of insects in the transmission of leprosy has received much investigation in recent years without any one insect being especially incriminated. Flies can carry numerous lepra bacilli

from ulcerated skin lesions to the skins of neighbours or to food, indicating the advisability of putting a stop to markets being frequented by begging lepers as is so frequent in the East. Acid-fast lepra-like bacilli have also been detected in the alimentary canals or faeces of insects fed on lepers, as shown by the following recorded data :—Bed bugs have yielded positive results in 9·9 per cent. of 302 bugs fed on lepers, and in one per cent. of those caught on them, while E. C. Long has recorded the development of leprosy in a man in South Africa within a year of his having slept in a bug-infested hut recently vacated by a leper, although he had never lived in a house with one. With a single exception investigators have found under one per cent. of mosquitos fed on lepers to harbour the bacilli. The *Acarus scabiei* of itch has been suspected by Heiser and by Mugliston to play a part in leprosy infection, while lice have also been accused.

*Explanation of Relation between Humidity and Leprosy.* I may now return to the relation between high humidity and temperature and leprosy rates which I showed in the first lecture, and for which I have suggested the following explanation. In the first place, as the leprosy bacillus has not yet been cultivated with certainty, it presumably lives but a short time outside the human body in the absence of any known animal or insect carrier, so that the absence of leprosy in very dry, hot countries may be explained by those conditions being most unfavourable for the extracorporeal survival of the leprosy bacillus ; while in hot, damp climates it will find more favourable conditions, especially if it gets on to the skins of neighbours of an infective leper. Such climates also greatly favour insect life, while the frequency of the first noticed lesions on the exposed parts of the body and extremities is easily explained on the ground that they are subject to frequent insect bites, affording minute lesions in the skin through which the lepra bacilli may gain access to the dermal connective tissues and nerve endings so favourable for their multiplication ; the presence of humidity and numerous insects thus favour infections from lepers living in the same house or in close association with the healthy, these being, as I showed in the last lecture, the essential conditions under which the disease spreads. Such an explanation also affords a good basis for the study of prophylactic measures, to which I must now turn.

## THE PROPHYLAXIS OF LEPROSY

Hitherto, the prophylaxis of leprosy has practically been summed up in the one word segregation. The first isolation laws are probably those of Moses; drastic measures were enforced in the middle ages in Europe, including deprivation of civil rights, divorce, isolation in leper houses, wearing a special garb, prohibition of entry into inns, churches and bakehouses, touching or giving anything to children, washing in common fountains, eating or drinking with any but a leper, speaking in the streets except in a whisper, etc.; all highly effective measures against a disease now known to be rarely acquired except by prolonged close contact with a leper. It is not of the highly contagious nature it was thought to be in ancient times and by some savage races to-day, who are recorded to have tried to control leprosy by killing all young lepers in Nigeria, or all ulcerated ones as in Nyasaland and Fiji, burning them alive in Sumatra, drowning them in Western India, etc. Isolation of advanced lepers in special villages has been reported from Central Africa, Madagascar, Indo-China, China and parts of India such as the Garo Hills in Assam, while even in Spain lepers are said to be driven into the mountains and deserts: so great is the fear of this mutilating and disfiguring disease, which still allows of severe segregation measures necessitating life-long isolation in most cases, being sanctioned by public opinion, such as would not be tolerated against other diseases with such a protracted course. The whole policy of segregation against leprosy requires careful reconsideration in the light of our present knowledge of its epidemiology, as a preliminary to which the results hitherto obtained by the measure must first be reviewed, beginning with those under the most favourable conditions in European races in the temperate zone.

*The Norwegian System.* As the greatest obstacle to segregation is the natural tendency of the sufferers to hide their affliction, the essence of the humane Norway system of segregation is to provide comfortable hospital conditions to attract the lepers, and to resort to compulsion as little as possible, that measure only being applied to indigents during the early years. In 1856, when the measures were commenced, there was good reason to believe that leprosy was increasing in the country, 2,833 cases then existing, or 1·91 per mille, six times the present official rate in India, and

only 235, or 8·3 per cent. were in leper institutions. Careful records were kept, while newly discovered cases were entered in the year in which their symptoms appeared, and after a time Hansen and Looft were able to report that the decrease in different districts was in proportion to the numbers isolated, and that the new cases arising in any area were in proportion to the number of unisolated nodular infective cases forming foci of infection. Owing to the favourable results obtained, the laws were strengthened in 1878 and again in 1885, when all lepers were isolated at public expense, and local authorities had power to remove lepers from their houses, unless they could be isolated at home under satisfactory conditions and close supervision. The results are shown in Fig. 1, which I have

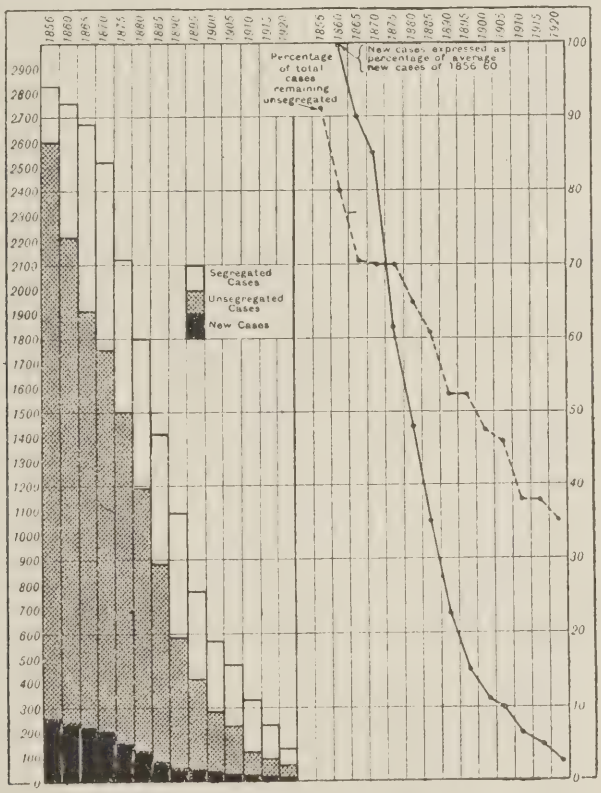


Fig. 1. Chart illustrating segregation in Norway.

prepared from the official figures, the latest having been kindly supplied to me by Dr. H. P. Lie (who succeeded Dr. Hansen in

charge of the work), the average number of new cases, and of the segregated and unsegregated being shown for each five-yearly period, while to the left the curves of the percentage of new cases, as compared with the average number from 1856-1860, and the percentage of unsegregated lepers in each five years are also given, showing a reduction of the total cases to 160 in 1920, while in 1923 there were only 140, or 5 per cent. of the original number in 1916-20 ; the new cases were only 2·7 per cent. of those in 1856-60, although it was not until after 1890 that over half the total lepers were isolated, these cases, however, including a much larger proportion of the infective nodular types. An interesting feature of the Norway experience was that five out of six of the newly-discovered cases dated their first symptoms back for three or more years, during which they had opportunities of infecting their households before they were discovered and isolated (a difficulty which is still greater among backward tropical races), and it accounts for the slow, if sure, effects of the measure, which is one of the most successful yet recorded, and clearly establishes the value of efficient segregation where it is practicable.

In SWEDEN compulsory notification and the provision of hospital accommodation in the affected central Helsingland and Dalecarlia districts led to a fall from the maximum number of 103 cases in 1873 to 89 in 1907 and 37 by 1923, a reduction of 65 per cent., the provision of comfortable conditions in the leper institution enabling a high proportion of the cases to be isolated without compulsion.

In ICELAND there were 226 cases, or 3 per mille in 1896 ; the leper hospitals, which had been closed in 1848 on account of the non-contagionist views of Danielssen, were reopened through Dr. Ehlers' influence in 1897, who also got a law passed prohibiting children under fifteen years living in the same house with a leper—a measure which would be of great value in this country if it could be applied to open infectious cases of tuberculosis. The result was that leprosy declined to 67 cases and 0·78 per mille by 1920, or just one-fourth of the rate twenty-four years before, fully justifying the steps taken. I am indebted to Professor Monrad-Krohn of Christiania for the recent figures of these three countries.

In MEMEL compulsory segregation and search for contacts were enforced in 1893, when there were 25 cases and the disease was still

increasing ; a decrease set in in 1898, by 1908 only 15 controlled cases remained, and by 1914 the outbreak had been stamped out : these results confirm the value of humanely conducted segregation in European countries. Further, in the New Brunswick province of Eastern Canada an old endemic focus dating from 1815 among the French was controlled, with the aid of the Catholic priests, by a voluntary system of isolation, and the cases slowly decreased from a yearly average of 32 in 1875-79 to 20 in 1910-14, and with more active measures fell to 12 in 1920-22, including a few from other parts of Canada.

*Segregation in the Sub-Tropical Zone*, 40° to 23½° F. In countries of this zone with moderate temperature and rainfall, leprosy rates are mostly low. CYPRUS in 1878 had over 150 known lepers ; in 1891, compulsory notification and segregation were introduced ; in 1901, there were 125 cases, 0·57 per mille, and in 1921 only 74, or 0·23 per mille, a decline of 60 per cent. The 1921 report states that there is now 'a good prospect of stamping out the disease in Cyprus before very long.'

The AUSTRALIAN COMMONWEALTH mostly falls in this zone, except the north of Queensland and the little-inhabited north coast, and compulsory segregation has been enforced for some time ; leprous Chinese and Oceanic islanders are repatriated, Europeans being retained until bacteriologically free for several years. Queensland had 78 lepers in 1910 and 50 in 1921, while New South Wales has had from 24 to 20 with a yearly average admission of three during the last fifteen years up to 1922 ; the remaining colonies have only isolated cases. A single institution might well care for all the cases in the Commonwealth.

SOUTH AFRICA presents a much more difficult problem with its large native population, and records of the admissions to the Robben Island settlement since 1845 show 20 to 30 yearly up to 1886, while immediately after the introduction of compulsory segregation in 1892 the yearly admissions rose to 294, and averaged 107 from 1894 to 1905 ; as late as 1907, Mackie reported that only 23 per cent. of the lepers registered that year could be accommodated in the asylums, and the recorded rates per mille for Cape Colony rose from 0·41 in 1891 and 1·02 in 1895 to 2·21 in 1907, clearly showing that in the absence of efficiently enforced segregation the disease was slowly increasing.

Recently the Union of South Africa has provided additional accommodation, and in 1923 there were 2,501 lepers isolated in several asylums, including the 693 bacteriologically negative cases since released as uninfective. In Basutoland, Long reports that the spread of the disease has been controlled by the active segregation measures enforced there in recent years.

*Segregation in Tropical Countries.* Still greater difficulties are met with in humid tropical countries with backward populations, although in some restricted areas with small numbers of lepers, promising results have occasionally been obtained ; these are best illustrated by the WEST INDIAN data I recently collected from the colonial reports, which are of especial interest,—the colonies who did not carry out segregation furnishing admirable controls, as shown by the following data :—

Jamaica began segregation in 1896. In 1891, there were 450 lepers, 1·23 per mille, and in 1921 only 319, 0·35 per mille, a decrease in twenty years of 52 per cent.

British Guiana began segregation in 1905. In 1891 there were 353 lepers, 1·23 per mille, and in 1921, 247, 0·83 per mille, a decrease of 42 per cent.

Trinidad only began segregation in 1915. Lepers in 1871 numbered 102, 0·93 per mille ; in 1891, 225, 1·12, and in 1921, 526, 1·50, an increase of 81 per cent.

Barbadoes had no segregation up to 1922. Lepers in 1901, 230, or 1·21 per mille, and in 1921, 164, 1·05 per mille, an increase of 48 per cent.

St. Kitts, with no segregation, had 112 lepers, 2·44 per mille in 1891 and 100, 2·60 per mille.

Of the smaller islands St. Lucia and Granada, without segregation, showed no change in the leprosy rate per mille, while St. Vincent, with segregation of indigent lepers, showed a decrease from 57 in 1881 to 30 in 1911 and 19 in 1921.

Thus all the colonies who relied on voluntary isolation in asylums of a few advanced cases showed either an increasing number of lepers or a stationary condition, while the island colonies which carried out compulsory segregation showed a great reduction within two or three decades ; thus affording a striking object lesson to other colonies.

FIJI commenced compulsory segregation in 1911 on an island settlement, where the numbers isolated increased from 40 in 1911 to 351 in 1920, and are now being treated efficiently by Dr. Harper, but it is too early to judge of the effects of these measures. Penrhyn Island has partial segregation, and only 27 cases in seventeen years. In the MALAY STATES a Leprosy Prevention Ordinance was passed in 1893 and leper asylums were gradually opened; the average yearly admission increased steadily from 150 in 1895-1901 to 174 in 1912-16, but fell to 152 in the five years up to 1921, indicating that a decline of the disease has now set in. CEYLON passed a leper ordinance in 1901, but deficient accommodation has prevented its efficient enforcement.

In our TROPICAL AFRICAN COLONIES, lack of funds has prevented anything material being done, except in Tanganyika, where there are 39 leper villages with land to cultivate, making many of them self-supporting.

In MADAGASCAR many lepers are isolated in settlements under missionary care, but I have not been able to get any information on the results yet obtained.

Two important trials of segregation in backward tropical areas under most difficult conditions remain to be considered, as they have important lessons.

In HAWAII, as early as 1865, a leper hospital was opened near Honolulu for the treatment of the earlier cases, and the famous leper settlement where Father Damien fell a victim to the disease after many years of invaluable work, was formed on the Molokai peninsula; but in spite of an expenditure of 4 per cent. of the total Island revenues the conditions were bad, resulting in the inevitable hiding of cases as long as possible, so that over 90 per cent. were of several years' standing when first discovered. Moreover, political influences caused great variations in the enforcement of the law, and very little effect was produced on the prevalence of leprosy, until in 1891, American influence greatly improved the conditions, since which the rate per mille has fallen from 13·5 in 1890 to 2·16 in 1919; during the last few years effective treatment has been introduced with favourable results, which I shall deal with in the next lecture.

In the PHILIPPINES the Americans, under Dr. Victor G. Heiser, inaugurated, under difficulties, a great effort by means of compulsory

segregation to stamp out leprosy, and opened the Culion settlement in 1906, regarding the results of which contradictory statements have been published. Thanks to the kindness of Dr. H. W. Wade, chief medical officer, in supplying me with the official figures to date, I have been able to work out Fig. 2, the upper curve of which shows the yearly (continued line) and three-yearly (broken line) average admissions, while the lower curve shows the yearly total Culion lepers (continued line) and the average three-yearly number corrected to the average mortality of the whole period (broken line).

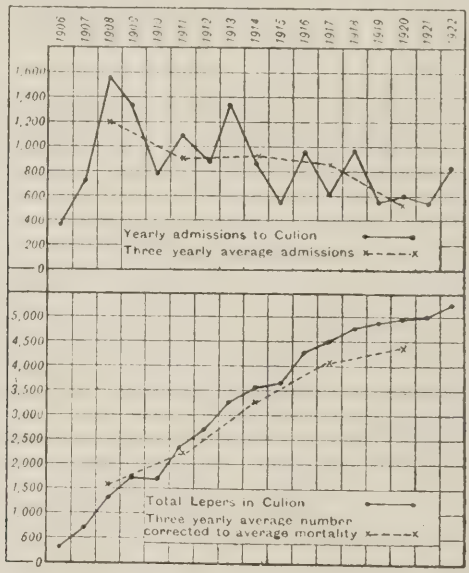


Fig. 2. Chart illustrating the result of segregation in the Philippines.

The admissions naturally show the highest rate in the first three years after the opening of the settlement, the second three years show a steady rate, the third a slight fall, and the three years 1919-21 a marked fall in the admissions, while 1922 shows a rise due to the satisfactory reason that numerous lepers voluntarily declared themselves to obtain the benefit of the improved treatment now available. The lower curve shows that the total number in the colony is still rising, although at a gradually less rapid rate, the flattening-out of the curve being especially marked for the years 1918-21, indicating that the peak of the curve was nearly reached, apart from the new factor introduced by the attractive power of the

new treatment. In view of the comparatively short duration of the measure, and the immense difficulties in collecting the lepers from the numerous islands of the Philippine group, this result cannot be considered unsatisfactory, although not coming up to the sanguine expectations with which it was started, and with the assistance of the American doctors now applying the improved treatment, still better results are likely to accrue within the next decade or two.

*Failures of Segregation and their causes.* In addition to the foregoing examples, many failures of attempted segregation are on record, which have their lessons, and they may most conveniently be classified in accordance with the causes of their lack of success.

Perhaps the most common cause of failure is vacillating policy, such as in French Guiana, where betweeen 1823 and 1891 no less than twenty administrative alterations in policy were promulgated, rendering continuous application of efficient measures impossible; Hillis and Neal record very similar conditions in British Guiana. In New Caledonia, the French first ineffectively tried isolation of the lepers in five villages, later in a central asylum on Art Island, which had to be given up for financial reasons, and then reverted to forty-two small leper villages, which failed owing to lack of authority of the native chiefs, although in the Marquesas Isles this plan is said to have been successful with the aid of gendarmes. Political influences prevented effective enforcement of leper laws in Louisiana and Algeria as well as at first in Hawaii.

Defective administration has been another frequent cause of failure, the worst instance of which is probably that of Crete, where Ehlers showed that under Turkish authorities the leper occupants of the four leper villages outside the four main towns not only begged in the cities, but the more successful of them bought their houses in the leper villages, and then let them to healthy persons while they went on begging tours through the island, and some of those who took their houses contracted leprosy; the so-called segregation being in reality an ideal method for spreading the disease: yet Hutchinson quoted this as an example of the failure of segregation. Deficient accommodation, such as obtained a few years ago in South Africa, also comes under this heading.

These examples will suffice to show the great difficulties encountered in enforcing efficient segregation in many tropical countries,

mainly due to the impossibility of discovering and isolating the earlier cases as long as we have no effective treatment of the disease. Moreover, the numbers of lepers in China, India and Africa make general segregation quite impracticable at the present time, if only on account of the utterly prohibitive expense, so the successful Norwegian system cannot be applied to any large proportion of the world's lepers without the addition of some means of attracting the early cases and of dealing with them by some simpler, cheaper, and more effective method than life-long isolation.

Improved treatment as an aid to prophylaxis is the essential requirement, and in order to enable me to suggest a policy for reducing the incidence of leprosy I must here anticipate the main conclusions I shall come to in my next lecture regarding the present position of the improved treatment, which will then be fully dealt with, and may be summarised in the following statements :—

1. Early cases of leprosy are now coming forward voluntarily for the new treatment in Korea, the Philippines, Hawaii, India, and elsewhere in numbers formerly unknown.

2. Muir has now shown that the majority of these early cases are not infective, so may safely be treated in out-patient clinics at small cost, and that nearly all early cases lose their symptoms within a few months—the few bacteriological positive ones become negative and consequently no longer a danger to their households, while a fair proportion of later cases also clear up under prolonged treatment.

3. When a considerable proportion of the early cases can be thus cleared up, and members of their households examined repeatedly to detect and treat, on its first appearance, those developing the disease, the number of foci of infection in the houses will rapidly be reduced and the occurrence of new cases and the incidence of the disease will steadily fall.

By combining this new and hopeful measure with the isolation of as many as possible of the more advanced infective cases in special hospitals, sanatoria on the lines of tuberculosis hospitals, and colonies with land to cultivate and with the provision of the best treatment, a far more rapid decrease of leprosy will be brought about than ever previously possible. The Honolulu results of the last three years, which I shall deal with in my next lecture, indicate that, if they are maintained for another seven years, the total number of known

lepers in those islands will be reduced by nearly one-half within a single decade, or three times as rapidly as under the successful segregation system in Norway.

## THE PRINCIPLES OF MODERN LEPROSY PROPHYLAXIS

The best prophylactic measures to adopt, necessarily vary widely under the conditions prevailing in different countries and climates, so that only the general principles can be laid down ; these can most conveniently be considered first as regards areas in which compulsory segregation is adopted with a view to rapid reduction of the disease, and secondly, as regards the measures which are possible where compulsory methods are impracticable.

*Legalised Compulsory Measures Should Include* :—

1. Compulsory notification of lepers by both medical men and laymen under penalties, as recommended by the Paris Academy of Medicine in 1914, and enforced in Sweden, Iceland, the United States and some of our West Indian Colonies.

2. Examination and admission to institutions of the lepers by an expert medical board, which should also deal with cases recommended by the institution authorities for release as no longer infective, as in Honolulu and the Carville leper colony of the United States.

3. Compulsory isolation, with the provision of the best treatment available, as laid down in a resolution of the 1923 Strasbourg International Leprosy Congress, and retention until the patient is proved by repeated bacteriological examinations to be free from all infectivity, any released cases being examined at fixed intervals for several years, to detect and deal with possible relapses, as in the United States, Hawaii and the Philippines.

4. The examination of all household contacts of discovered lepers, about every six months for from three to five years, to detect new infections in the earliest and most curable stage of the disease. These periods are based on the results I have recorded of a study of 84 collected cases indicating an average incubation period of two to three years, while in 65 per cent. the time did not exceed three years and in 81 per cent. was not over five years ; these figures show that four-fifths of new infections would be detected within the longer period.

5. The separation of the children of lepers from their parents as soon after birth as possible, and the prohibition of healthy children and adolescents from living in the same house with a leper, as in Iceland, where an unusually rapid decrease of leprosy has recently taken place. The prohibition of leper children or teachers in schools for healthy children is also essential, but has frequently been neglected.

6. The separation of the sexes in leper institutions, except husbands and wives beyond the child-bearing period, as enforced in Australia and the United States, the neglect of which measure in Hawaii and formerly in the Philippines, as already mentioned, led to infection of a large proportion of the children born to the lepers. In Panama an effective compromise has been adopted, worthy of imitation where complete separation of the sexes has not been found practicable, by only permitting marriages when the male leper applies for and submits to the simple operation of sterilisation by bilateral section of the vas deferens, which does not affect marital relationship, while preventing the procreation of children. The Calcutta Leprosy Conference of 1921 urged separation of the sexes to prevent the calamity of a healthy partner becoming infected while the other was recovering under treatment.

7. Countries adopting such stringent measures to eradicate leprosy must have legal powers to protect themselves from re-infection by repatriating all immigrants arriving with or developing leprosy within the longest known incubation period, which in exceptional cases has been known to extend to twenty or more years.

8. The prohibition of lepers from engaging in dangerous occupations, such as the preparation or sale of foods, clothing, cigars or cigarettes, the care of children or sick, domestic employment, midwifery, the barber's trade, prostitution, etc., is essential in all leprous countries, although not yet enforced even in India.

Home isolation was permitted in Norway, mainly in the less infective nerve forms, where the accommodation permitted the leper being provided with separate room, cooking and table service, bedding, clothing, washing, etc., under close medical supervision; but this measure failed to prevent infections in Roumania and South Africa, and should not, in my opinion, be permitted when

there are any children in the house or when the case is an infective nodular one.

The treatment of early uninfective cases, in which no lepra bacilli can be found by repeated examination in the skin or nasal mucous membrane, as out-patients in special hospital or dispensary clinics, which Muir is rightly advocating as the most practical measure in such countries as India, where compulsory segregation is impossible, may also have to be considered where compulsory methods are enforced, and this measure appears to be worthy of trial to attract the early more amenable cases, with the proviso that any patients not regularly attending until released by an experienced medical officer will be liable to be segregated.

*Prophylaxis where Compulsory Segregation is Impracticable.* It will be remembered that in the early years of the Norwegian work, and throughout the successful campaign in Sweden, many lepers were isolated on a voluntary basis by supplying good hospital accommodation for them. Now that a far more effective treatment is already attracting many earlier cases of leprosy to hospitals, much more can be done towards isolating lepers on a voluntary basis than formerly, and the measures best suited to extend this policy remain to be considered ; the following system has been advised by the Calcutta Conference and endorsed by the Government of India, and is gradually being brought into operation in the different provinces as funds permit.

*Leper Asylums and Colonies in India.* At present there are some 9,000 lepers segregated in India, some of the large cities having prison-like asylums with high walls surrounding small spaces, but most of the cases are in country asylums administered by the Mission to Lepers and other missionary bodies, with financial assistance from the Government, ranging from the well-organised Purulia institution with 700 lepers and ample ground for cultivation, to a small house with ten or so lepers. Some three-fourths of the inmates are advanced anaesthetic mutilated cases, for the most part uninfective and quite unamenable to treatment, whose isolation has little or no prophylactic value, while, unfortunately, in many of the asylums regular up-to-date treatment is not yet available.

Under the new system now being introduced, large institutions are being provided with ample land for cultivation, work on which

is as beneficial to lepers as exercise is for the tuberculous, and will also enable most of the required food to be home grown. Only infective cases should be admitted, under an efficient administrative and medical staff providing the best treatment and care, hospital accommodation for advanced and complicated cases, and a separate area, on a cottage or other convenient system, for the earlier ones to save them being repelled by the mutilated class, and another separate part for healthy children, the sexes being separated on the lines already mentioned. Patients becoming bacteriologically negative should be kept in a convalescent section until passed for discharge as at Carville, or separate small colonies with land provided for them as at Purulia, where there is already a village of recovered lepers, something on the lines of the Papworth tuberculosis colony near Cambridge.

These leper colonies, or, as they might well be called, sanatoria, will be complementary to the hospital and dispensary clinics already mentioned, as any advanced cases unsuitable for treatment at the latter, and likely to repel the attendance of the early amenable class it is so important to attract, should be sent to the colonies for prolonged treatment and care. Where the clinics are opened before colonies are available, the difficulty may be dealt with by arranging for the early cases to attend on different days from the more advanced and infective ones.

As the proposed well-equipped and staffed colonies will be expensive institutions to maintain, I regard it as most essential that advanced mutilated, little or not infective, begging anaesthetic cases should not be admitted to them, as treatment will be useless, and its apparent failure depressing to the more amenable nodular cases, while it is a waste of good money to isolate cases who are no danger to the community, and should be looked after by their relatives. The good example of South Africa in releasing from the asylums, the 693, or 28 per cent. of the total of such uninfective lepers is worthy of imitation in other countries without unlimited funds for dealing with leprosy.

In our tropical African colonies the difficulties of dealing with the leprosy problem are even greater than in India, although the same principles are applicable, beginning with the provision of leprosy clinics for treatment until the confidence of the patients is gained,

when the more infective cases may be induced to live in leper villages, which are already a familiar measure among many tribes. Sir Hugh Clifford, in Nigeria, has started some leper villages into which only lepers are admitted, allowing them to retain their self-respect, while all children born to them are removed from danger of infection by being sent to healthy relatives as soon after birth as possible: an admirable system which is worthy of wide adoption under similar circumstances.

Such, in brief outline, are the conclusions I have come to on the vexed question of leprosy prophylaxis based on three years' minute study of the literature, and adapted to take full advantage of all the recent advances in our knowledge of the epidemiology and treatment of this terrible disease. Many decades of patient work lie before us, but I feel it is high time we made a serious attempt to utilise the means we now possess of reducing leprosy in our Empire; regarding which our American cousins have recently set us such a good example in Hawaii and the Philippines.

## Lecture III. THE TREATMENT OF LEPROSY

*Preliminary Considerations.* Leprosy presents many difficulties in estimating the effects of any given treatment, due to differences in the types of the disease, the very chronic and variable course it runs with sudden exarcerbations, sometimes followed by temporary improvement. Moreover, in the anaesthetic variety there is a tendency for the progress of the disease to cease and even for the infection to die out, although rarely until after serious permanent crippling of the extremities has been produced through nerve destruction rendering treatment ineffective. On the other hand, when ill-nourished begging lepers are cared for in an asylum, considerable improvement may ensue without drug treatment; for which reasons short trials of drugs in a few cases have little value. Even more difficult is the estimation whether great improvement, even amounting to disappearance of all active signs and infectivity of the disease under prolonged treatment, will prove permanent or not: the analogy with tuberculosis being here very close, for it is impossible to say whether any living bacilli which may produce a relapse some years later remain quiescent in the tissues. The spontaneous

disappearance of very limited anaesthetic patches has also very occasionally been met with, but natural recoveries of the nodular forms rarely if ever occur, although such cases may sometimes develop nerve symptoms, which gradually predominate over the skin ones, forming mixed cases of leprosy. In short, there is a nicely balanced struggle between the invading bacilli and the tissues of their host, liable occasionally to be turned in favour of the latter, which necessitates caution in judging of the effects of any remedy without long observation on a series of cases, but which also affords good hope of curative measures being discovered by patient research. These now appear to be within sight in certain soluble derivatives of chaulmoogra and other oils, before dealing with which a brief review of other methods which have afforded great temporary improvement and a few apparent cures will be of interest and throw some light on the conditions favouring recovery.

*Mineral Preparations.* Mercurial preparations have been recommended from the days of Pjetursson in Iceland, in 1769, to Radcliffe Crocker in 1896, but have not recently met with favour. Arsenic was advised by Danielssen in Norway, while atoxyl, arrhenal, salvarsan and, recently, eparsono have been advocated, chiefly by French writers, with variable and uncertain results. Antimony intravenously has recently been advised by F. W. Cawston, and appears to be of some value in healing leprotic ulcers, although workers in Culion and elsewhere have not been able to confirm the original claims made for this form of medication. Cyanocuprol has been advised in both tuberculosis and leprosy by Japanese workers.

*Iodine* has greater claims; Danielssen in 1886, and others, using the iodide in the treatment and diagnosis of leprosy, observed febrile and local reactions in nodular lesions, with the disappearance of old and the appearance of new nodules, as well as increased discharge of lepra bacilli in the nasal mucus of diagnostic value following its use. Iodoform and europhene injections have been advised by Neisser and others, while Clegg and Hollmann obtained interesting febrile reactions after the inhalation of 15 to 30 minims of ethyl iodide, and Marchoux and Bourret in 1909 observed that during reactions following iodides, large numbers of leprosy bacilli lose their acid-fastness and are destroyed; so the drug may be of

value in conjunction with other remedies, although by itself it has failed to produce lasting beneficial results in leprosy. Ichthyol, guiacol, strychnine, etc., have also had their advocates.

*Local treatment* has been advocated, especially by G. Unna, including the destroying of nodules by shaving off with a razor, applying carbolic acid, hydrochloric acid, caustic potash, the cautery, etc., but the claims to eradicate the disease in early cases by such measures have not been substantiated. Tincture of iodine, carbon dioxide snow, ethyl chloride (Lie), trichloracetic acid, mineral baths, X-rays, radium and electric currents have all had their advocates: the multiplicity of remedies indicating that no really satisfactory one was available up to very recent times.

*Serums* against leprosy have been prepared by Carrasquilla in Colombia, in 1896, by injecting horses with the blood of lepers, while later others, with more reason, but without success, injected animals with antigens composed of juice of nodules, containing lepra bacilli while Dyer in America tried antivenomous and normal horse sera with no result.

*Vaccines* made from various acid-fast organisms have been extensively tried with temporary good results in some cases. Tuberculin produces well-marked reactions in leprosy, but with large doses of Koch's original form more harm than good was done, an analysis of fourteen papers up to 1892 showing that slight improvement was only claimed in one trial, but in 1896 Arnaud saw disappearance of nodules and improvement continuing for two years following a severe reaction: a case that once more illustrates the great benefit occasionally following upon violent reactions induced by very different lines of treatment. In 1904-5 Lie of Norway recorded post-mortem evidence to prove that reactions may be obtained with tuberculin injections in lepers who were quite free from lesions due to the tubercle bacillus, but he failed to get benefit in lepers he treated with small doses of tuberculin, although in 1909 Baber reported remarkable improvement in several cases after the use of tuberculin combined with chaulmoogra oil.

Nastin is essentially another non-specific vaccine made by Deycke by dissolving a fatty substance, extracted by ether from an acid-fast streptothrix, in benzoyl chloride, the injection of which, in leprosy, also produced febrile and local reactions, sometimes

followed by considerable improvement, very promising results being reported for a time by Deycke and others, until a four years' trial in British Guiana, initiated by the discoverer himself, and reported on by Wise and Minett in 1912, showed that general reactions with softening and absorption of the nodules only occurred in 3·5 per cent., of the cases treated and in the remainder early slight general improvement during the first three months was followed by retrogression, and the patients got steadily worse ; their conclusion from prolonged study of 244 unselected cases was that nastin produced only ' a slight temporary check during the first six months of treatment, but otherwise the natural course of leprosy continued unchanged.' Such temporary changes accounted for the improvements shown in Deycke's tables, while subsequently Minett found that injections of benzoyl chloride alone produced precisely the same effects as Deycke's nastin-B itself.

Vaccines from supposed acid-fast bacilli of leprosy have been made by Rost, Williams, Clegg and others from cultures obtained from cases of leprosy, which, however, Walker has recently shown cannot be distinguished from the smegma bacillus, so are also non-specific acid-fast bacilli, but their use has undoubtedly been followed by local and general reactions, as with tuberculin, followed in some cases by great improvement, only too often of a temporary nature. Harm, however, can also result ; Rutherford, in twenty carefully noted cases, found that the deterioration exceeded the improvement, while Clegg's bacillus gave negative results in Honolulu : the effects of this treatment on the whole are, therefore, disappointing.

Vaccines made from excised lepra nodules, containing enormous numbers of lepra bacilli, have also produced some benefit, but this plan has obvious limitations, especially where the disease is not common. Nevertheless, as will appear presently, the reactions produced by the various acid-fast bacillary vaccines may make them of some value in combination with other lines of treatment.

*Chaulmoogra oil* is an old Indian remedy, which one writer thinks was referred to in the ancient writings of Susruta as ' tuvaraka,' but was introduced to western medicine by Mouat in a paper in Vol. I of the *Indian Annals of Medical Science* of 1853-4, and was made official in the Pharmacopoeia of India in 1868 and in the Indian

and Colonial addendum to the British Pharmacopeoia in 1901. There has been a good deal of confusion regarding the origin of the oil, which was for many years erroneously described as being derived from the seeds of *Gynocardia odorata*, until in 1901 Sir David Prain showed that it came from those of *Taraktogenos kurzii* (King) growing along the banks of the rivers of Assam, Chittagong and Burma. It has since been found that various species of *Hydnocarpus*, the most important of which are *H. wightiana* of Southern and Western India, and *H. anthelmintica* of Siam, Indo-China and China, all contain the same active unsaturated fatty acids as *Taraktogenos*, so that it will be convenient to include the oils of both these genera, but not that of *Gynocardia odorata*, under the term chaulmoogra oil.

Much work has been done on the chemical constitution of these oils, Moss, as early as 1879, separating the lower melting point acids under the term gynocardic acid, while Power and Gornall in 1904 and the following years separated first the highest melting point (68° C.) chaulmoogric acid, established its chemical formula and made a number of compounds, including methyl and ethyl chaulmoograte, and in 1905 Power and Barrowcliff isolated from *H. wightiana* oil, both chaulmoogric and a lower melting point acid (60° C.), named by them hydnocarpic acid, all of which I shall have to refer to again.

Chaulmoogra oil taken orally has long had a reputation in the treatment of leprosy, but has the great disadvantage of being so nauseating that few patients can take sufficient to do more than retard the progress of the disease. The best results have been obtained by Ralph Hopkins, with fifteen years' patient work in the Louisiana Hospital, with 30 per cent. improved, 5 per cent. progressed and 71 per cent. died, of 88 advanced cases ; 17 per cent. cured, 4 per cent. lesions disappeared, 48 per cent. improved, 5 per cent. progressed and 12 per cent. died, of 82 incipient cases, showing that only in incipient cases was very material benefit obtained; but demonstrating that the oil had a definite value in leprosy.

A Chinese method of giving the fresh *Taraktogenos kurzii* nuts orally combined with hemp and another Chinese drug has recently been reported by E. A. O. Travers of the Kuala Lumpur Leper Asylum in the Malay States, to have cleared up the symptoms of a certain number of cases of leprosy, while the nuts are very

cheap and readily taken by women and children. He is now, at my suggestion, trying *Hydnocarpus wightiana* nuts, for Reed states that these keep fresh for months if dried, and they can be obtained at about three shillings for 80-lbs. weight, from the Ernakalum Trading Co. of Southern India, being much easier to get than those of *Taraktogenos*. Competent botanists assure me that the *Hydnocarpus wightiana* tree is likely to grow well in any hot, moist climate, with a good rainfall, that is in just those districts where leprosy is so common, and I hope to be able to get the seeds of this species widely distributed before long, if the Malay results are confirmed by further experience.

The importance of the above methods of administration is that they have led to the discovery of more efficient preparations derived from the oils, the evolution of which must now be described.

Gynocardic acid, consisting of the lower melting point fatty acids of the oil, has been used orally since 1891, together with sodium and magnesium gynocardates, with apparent benefit in some cases. I administered gynocardic acid to a few lepers during the first decade of the present century, and came to the conclusion that it was much less irritating to the stomach and more effective than the whole oil, one European patient taking up to forty grains a day for a year, with the result that a very extensive macular leprosy completely cleared up, although some nerve symptoms persisted. As early as 1912 I asked an important firm of manufacturing chemists if they could make for me some soluble compound of gynocardic acid suitable for injection, but unfortunately received a reply in the negative.

In 1911 Engel-Bey reported good results in a few lepers treated orally in Egypt with antileprol, made at his suggestion by separating the free acids of chaulmoogra oil and esterising them, while in 1913 H. Bayon gave this preparation both orally and intramuscularly.

Chaulmoogra oil intramuscularly appears to have first been used successfully by Tourtoules in Egypt, who reported apparent recovery in one case after 650 injections totalling 2,720 grammes in the course of six years, ending in 1899, while Hallopeau in Paris reported benefit in nine lepers treated with combined oral and intramuscular administration, although Castel in 1899 recorded pulmonary embolism in two cases. Jeanselme in 1911 injected

a mixture of chaulmoogra oil, camphor and guiacol, while in 1914 Victor G. Heiser reported from the Philippines 11 per cent. of apparent cures in a small series of cases treated for prolonged periods by intramuscular injections of a mixture of equal parts of chaulmoogra oil, camphorated oil and resorcin, and later Hopkins, McCoy and Hollmann recorded successes by this method, which thus constitutes an important advance.

*Sodium Gynocardate Intramuscularly and Intravenously.* As the result of Heiser's success with chaulmoogra oil intramuscularly, and of a personal visit from him in Calcutta in 1916, I renewed my attempts to obtain the active portion of the oil in a soluble form suitable for injection, and with the help of Dr. Chuni L. Bose, Professor of Chemistry, and later of the whole time assistance of Dr. Sudhamoy Ghosh, D.Sc., Edin., and with the financial assistance of the Indian Research Association, I obtained first sodium gynocardate, and subsequently similar compounds of the various fatty acids of chaulmoogra, cod-liver and other oils, and investigated their action in numerous cases of leprosy during the next four and a half years. The following results were obtained :—

Intramuscularly sodium gynocardate, prepared from gynocardic acid with a melting point of 37° C., was better borne by Indian patients than the whole oil, and although it produced local pain and induration it gave promising results. I next ascertained by animal experiment that it could safely and practically painlessly be injected intravenously with only very temporary giddiness in a 3 per cent. solution, a large medio-basilic vein allowing of weekly injections for upwards of a year, but when only small veins were available, as is often the case in women and children, irritation of the inner lining of the vein at the immediate site of injection might produce strictly localised and harmless obliterative phlebitis limiting the injections ; this irritation was only partially prevented by the addition of ½ per cent. sodium citrate to the solution. I next found that the sodium salts of chaulmoogra oil, fatty acids with melting points of from 49° to 51° C., containing both gynocardic and hydnocarpic acids, and which I called gynocardate of soda A, were more effective in leprosy than those of the lower melting point acids, while the salts of chaulmoogric acid itself were much less soluble and also less active. Eventually I came to the conclusion

that the salts of the whole of the fatty acids of *Hydnocarpus wightiana* oil, containing more hydnocarpic and less chaulmoogric acid that the oil of *Taraktogenos kurzii*, gave the best results, and this oil has since been used in Calcutta by Muir and by many other workers in the East in making preparations for injections in leprosy, the tedious and expensive process of fractionation being unnecessary now that these points have been established by careful investigations. Professor B. E. Reed of the Peking Union Medical College, has recently stated that *Hydnocarpus* preparations, mainly supplied from Calcutta, have found favour in Singapore, the Malay States, Burma and elsewhere, and he concluded that 'the antiquity of the records of *hydnocarpus*, the continuity of its use in many countries, its high chemical and therapeutic worth, give it a place of international importance.'

*Reactions with Destruction of the Lepra Bacilli due to Gynocardates and Hydnocarpates.* The subcutaneous injections of sodium gynocardate produced gradual improvement in leprosy cases, without the occurrence of any marked reactions in the affected tissues, but when I commenced to give the preparation intravenously a remarkable and hopeful phenomenon was observed, nearly always in rather advanced nodular cases with enormous numbers of lepra bacilli in the affected tissues. The reaction which took place is well illustrated by a coloured plate I published in 1919 showing inflammatory swelling and softening of the leprosy infiltrated lobes of both ears, while microscopical examinations of excised portions in such cases revealed only a few remaining typical rod-shaped acid-fast bacilli, together with innumerable acid-fast granules of disintegrated organisms, demonstrating that the inflammatory local reactions produced by this vegetable substance had resulted in the destruction within the human tissues of enormous numbers of the pathogenic organism, and opening up possibilities of an important advance in the treatment of this hitherto intractable disease. Fever for a day or two always accompanies such reactions, which may be induced by very minute doses of the drug, and very occasionally fever may persist for one or more months. This is accompanied by a softening of numerous nodules and a considerable degree of toxaemia resulting in prolonged debility, while a number of new skin lesions may appear in the form of slightly

raised red patches, just as occurs naturally, from time to time, in the more acute cases of nodular leprosy in what Muir calls the reactionary phase of untreated cases. As few bacilli are found in reaction lesions they may largely be due to inflammatory reactions excited in small deposits of lepra bacilli, which had not previously produced visible lesions while still quiescent. As a rule such reactionary lesions appearing during treatment clear up again rapidly, although occasionally some of them persist and the patient appears to be worse for a time, so it is now generally considered advisable to try as far as possible to avoid the more severe reactions by cautious dosage in the active second stage of the disease. Muir, however, finds that in the first stage of very limited lesions, as well as in the third quiescent stage, when the reactionary phase no longer occurs naturally owing to the establishment of tissue resistance to the toxins of the bacillus, the treatment may safely be pushed with beneficial effects. Considerable experience, both as regards the natural course of the different varieties of leprosy and also in the exhibition of the powerful remedies now available, is thus necessary to enable the best results to be obtained, which is, doubtless, the reason why some observers have failed to get good effects in their earlier attempts to use the new treatment.

Severe febrile and local reactions are, however, exceptional, steady improvement in their absence far more frequently ensuing, although careful observations in the wards of leper institutions enabled Muir to observe that slight rises of temperature, not noticed by the patient, nearly always occur in patients showing fairly extensive bacteriologically positive lesions, indicating the destruction of smaller numbers of bacilli of a beneficial nature. I was also able to demonstrate by repeated microscopical examination of small excised portions of nodules from the ear or other affected part, that in the entire absence of any reactions noticeable to the patient a gradual breaking up and diminution of the lepra bacilii was brought about by repeated injections of these preparations, accompanied by slow absorption of the nodules, until nothing but a few acid-fast granules could be detected; this stage, in turn, was soon followed by the entire disappearance of the bacilli from the tissues, as well as from the nasal mucus, rendering the patients apparently free from both all the symptoms and infectivity of the disease,

six to eighteen months usually being required to bring about this happy result in typical, but not extremely advanced, nodular cases.

In anaesthetic cases with nodular thickening of the ulnar nerves, I have also seen reactions consisting of temporary swelling of the nodules accompanied by severe pain, sometimes necessitating the use of morphine, but followed by subsidence and eventual great improvement of sensation in the area supplied by the affected nerve, while in less advanced cases steady, but usually slow, return of both sensation and muscular power occurred, together with eventual disappearance of the depigmented patches in various parts of the body. One of the earliest cases of this type, with foot drop greatly crippling him, lost all visible signs of the disease and became able to walk ten miles at a stretch. On the other hand, when nerve trunks of the extremities have been extensively destroyed by prolonged disease, and fingers and toes have been lost, or the typical claw hand with wasting of nearly all the intrinsic muscles has developed, complete restoration of function is obviously impossible, although a considerable degree of recovery of sensation and muscular power may take place when the disabilities are of recent origin, but not in the long standing crippled cases, so frequently seen in Indian leper asylums, with permanent destruction of extensive portions of their distal nerve trunks by irremovable fibrous scar tissue. Such wrecks of humanity may remain in a stationary condition for several decades before some intercurrent disease releases them from their misery, and the disease can only be prevented from reaching this incurable stage by effective treatment at an early period.

An even more remarkable and important phenomenon which I have observed in a very few bad nodular cases, who developed most prolonged and severe febrile reactions after even a single minute dose of sodium gynocardate, with great debility and inflammatory softening of extensive lesions, has been a steady improvement extending over many months, without any further treatment, and even complete recovery during the ensuing year ; of this type of case the following are examples : One of the worst cases I have seen of nodular leprosy of twenty years' duration, with extreme thickening of the skin of the face and extensive ulceration of both the ears and

the hands, had three months fever after the minute intravenous dose of 0·2 c.c. of a 3 per cent. solution of sodium gynocardate, followed by steady improvement without any further treatment, and at the end of a year only loose folds of skin remained at the site of the former facial nodules, and the ears and hands had completely healed. Another patient with a 'grog blossom-like' nose and raised red leprotic patches the size of the palms of the hands on his body, after two months reactionary fever following a few small doses of the same drug with great loss of strength, was given no further treatment except sodium morrhuate orally, although he was anxious to continue the injections. I sent him away for a change as soon as he began to pick up, and saw him again about a year after the reaction, when only slight pitting with some fibrous scarring remained at the sites of the former extensive lesions, sections of removed portions being quite free from acid-fast bacilli; complete recovery had thus taken place. Such cases are quite exceptional, but their occurrence at least indicates that the action of chaulmoogra oil soluble preparations cannot be explained solely by any direct destructive effect on the lepra bacilli: a point of great theoretical importance which I shall return to in the last lecture.

*Sodium Morrhuate and Sodium Soyate in Leprosy.* The destruction of the lepra bacillus after intravenous injections of gynocardates naturally led me to consider the possibility of inducing a similar change in the acid-fast bacillus of tuberculosis, and I consequently got Dr. S. Ghosh to extract the fatty acids from cod-liver oil and make a sodium salt for me, which I called sodium morrhuate. I found it made a clear solution almost unirritating both by subcutaneous and intravenous injection, so I next tried it by both methods in leprosy, and soon observed that it could induce by either mode of administration febrile reactions in leprotic tissues with destruction of the lepra bacillus, followed by similar improvement and ultimate disappearance of all signs of the disease. This will be evident from the coloured drawing I show you, in which six months' treatment with practically painless subcutaneous injections of a 3 per cent. solution of sodium morrhuate brought about the absorption of very numerous raised circinate red patches on all parts of the body of the patient, with disappearance of the lepra bacilli, leaving only lighter depigmented patches, although it should be

mentioned that he left off treatment against advice at this stage and I found a slight recurrence in one spot a year later.

As the value of chaulmoogra oil had previously been attributed to its possessing a closed carbon ring, a unique constitution for a fatty acid, the activity of sodium morrhuate in leprosy disproved that interesting theory, and led me to think that the proportion of unsaturated fatty acids as a class might be the most important factor influencing the therapeutic value of oils in leprosy. To test this theory I next selected some oils with a high iodine value, including soya bean oil and Japanese sardine oil, and had similar sodium salts of their unsaturated fatty acids prepared ; I found the latter to be irritating to human tissues, but the former, which I called sodium soyate, formed a clear and unirritating 3 per cent. solution suitable for injection either subcutaneously or intravenously. There was only time to try it in a few cases before I left India, in one of which an extensive, red, raised leprotic patch covering the whole of one cheek, together with smaller ones on other parts of the body, completely disappeared and the tissues became bacteriologically negative in the remarkably short period of six weeks ; this is by far the most rapid improvement I ever saw in a case of that degree, although the effects were much less rapid in the other cases, and as far as I know, this preparation has not since been tried on a sufficient scale to decide its precise value. The few tests I did, add yet another oil to those furnishing active preparations against the acid-fast bacillus of leprosy. In 1919, Dr. K. K. Chaterji, of Calcutta, applied my methods to nim oil, and reported great benefit in two lepers treated by ' margosates ' thus prepared. During the last two years, Muir has obtained active preparations on the same lines from linseed and olive oils, although he concluded that those prepared from the ' closed ring ' fatty acids of chaulmoogra oil were rather more effective in leprosy than the others, while preparations from saturated fatty acids had very little effect in this disease. This largely confirms my theory, and at the same time opens up an unlimited field of research on the many oils, which may furnish still more effective preparations, both in leprosy, and possibly also in tuberculosis.

*Results of Treatment with Gynocardates and Hydnocarpates and with Sodium Morrhuate.* In 1917, I reported twenty-six leprosy cases

treated for three or more months with sodium gynocardates and hydnocarpates with improvement in all, while eight of the twelve treated for a year or more had lost all signs of the disease, and added 'whether permanent results can be obtained only time will reveal.' In 1919, I recorded fourteen cases treated with sodium morrhuate, and the following table shows the results obtained in all the cases I had treated for three months and over, when I left India early in 1920, the sodium morrhuate series being shown separately.

TABLE III

| | Not Improved | Slightly Improved | Greatly Improved | Lesions all disappeared | Total Cases |
|---|---|---|---|---|---|
| Gynocardates and Hydnocarpates— 3 months and over ... ... ... | 1 | 9 | 20 | 21 | 51 |
| Gynocardates and Hydnocarpates— over 1 year ... ... ... ... | 1 | 1 | 2 | 9 | 13 |
| Sodium morrhuate— 3 to 12 months ... ... ... | 0 | 3 | 12 | 5 | 20 |

Thus, with the chaulmoogra oil preparations, in round numbers, 40 per cent. had cleared up completely and another 40 per cent. had so greatly improved that there was very good probability of their losing all signs of the disease with further treatment, giving 80 per cent. of good results, while of 13 cases treated for the sufficient period of a year or more, 9, or 69 per cent., had cleared up completely. The sodium morrhuate cases had been treated for shorter periods of from three months to one year and of 20 cases 12 had greatly improved and 5 had completely cleared up, the results being about equal to those with the gynocardates and hydnocarpates when the duration of treatment is taken into account. In only one very advanced nodular case of the total 71 cases had no improvement resulted, but it must be mentioned that as the cases represented all stages of leprosy, including some early ones, they were considerably more favourable than the average type met with in leper asylums.

As I pointed out in 1919, relapses occur in some cases which leave off the treatment as soon as the lesions have disappeared, and of 34 cases followed up since my report of 1917, one remained unimproved, 10 had further improved under continued treatment, in 5 the lesions

had now disappeared, 13 remained clear of symptoms and 5 had relapsed—the latter, all cases in Indians who had left off the treatment prematurely against my advice. These results clearly indicate the necessity of continuing injections for some months, or better still a year, after the disappearance of outward signs of the disease, as was only to be expected in a chronic affection due to a highly-resistant organism. Some of the relapsing cases cleared up again on resuming treatment. Further cases becoming stationary on hydnocarpate treatment, may proceed to clear up completely on using sodium morrhuate, illustrating the advantage of having more than one effective remedy.

*Disadvantages of the Intravenous Method.* The necessity of giving sodium hydnocarpate intravenously to obtain the best results is a serious disadvantage, on account of its irritant action on the veins, leading to obliteration, and the time consumed as compared with an intramuscular injection. Further, sodium morrhuate has been found to deteriorate in solution through oxidation, while it is troublesome and expensive to put it up in capsules to avoid change. Dr. Ghosh made some ethyl hydnocarpate for me, which I had not the time to try to any extent before I left India, and the next advance is due to American workers.

*Ethyl Ester Chaulmoogrates Intramuscularly.* In 1919 Dr. H. T. Hollmann and Professor Dean of Honolulu reported on the use of intramuscular injections of ethyl esters of the different fractions of the fatty acids of chaulmoogra oil, and confirmed my conclusion that the chaulmoogric acid fraction produced little effect, but that the lower melting point ones were more active. They obtained marked improvement in 17 out of 26 cases, improvement in 3, slight improvement in one and no improvement in 3, who had been treated for three months or less, very similar results to my earlier Calcutta ones. Their method had the great practical advantage that the ethyl ester could be injected in a pure fluid state into the gluteal muscles without much pain, enabling a large number of cases to be dealt with in a comparatively short time. This convenient modification has since been very generally adopted in many parts of the world with slight variations, Muir having introduced a very convenient formula, which in its latest form consists of equal parts of ethyl hydnocarpate (prepared from *H. wightiana*) and of pure olive oil,

with 4 per cent. double distilled creosote (E.C.O. mixture), his former E.C.C.O. mixture having also contained camphor (which J. G. Samson and G. Limkako at Culion found to be useless, although they confirmed the value of the addition of creosote), or 10 per cent. thymol (E.T.O.) may be substituted for the creosote. Muir advises injections of these mixtures either beneath the leprous lesions or intragluteally, or both, in doses rising by 0·5 c.c. at a time from 0·5 c.c. up to a maximum of 10 c.c. once or twice a week, the next dose being reduced if the temperature rises to 100° F. and remains up for more than 24 hours, while if any marked reaction occurs in the diseased tissue, injections should be stopped until it has completely subsided.

At my suggestion Muir tried giving iodide of potassium orally in daily doses of from ½ to 20 grains, and found this addition may induce reactions after ethyl hydnocarpate when absent without it, resulting in further improvement, and he has also used a vaccine of Kedrowsky's acid-fast bacillus with success for the same purpose: illustrating the principle that a variety of substances, that I showed in the historical review of former treatments have produced reactions in leprosy, may be of value to supplement the action of the new methods. Time does not permit me to go into further detail regarding the treatment of different stages of leprosy, which will be dealt with in a work on which Dr. Muir and I have been long engaged, and I must pass on to consider the results which have been obtained in other countries where the different remedies have been tested, the most extensive trials of which we owe to American workers.

In India I arranged, through the kindness of the Mission to lepers, for a trial of both sodium gynocardate and sodium morrhuate separately in thirteen leper asylums, and asked Dr. Muir to make an independent analysis of the results. 300 cases showed 72 per cent. improved and 32 per cent. greatly improved although the treatment had only lasted for from two to twelve months, and those treated for six months and over to a year gave 100 per cent. improved and 52 per cent. greatly improved, the more promising cases having been selected for treatment, while the two preparations gave almost identically the same results, establishing the principle that those from other oils than chaulmoogra may be effective in leprosy.

*Results with Ethyl Chaulmoograte and Hydnocarpates.* It is still too early to allow of anything like final conclusions regarding the precise value of the new treatment in leprosy, but the following extensive trials will afford some indications. During the last two years a great test was begun in the Culion settlement under Dr. H. W. Wade, who has kindly sent me a preliminary report showing that treatment was begun first in May, 1921, 500 cases being treated during the next two months, and gradually increased to 1,500 by April, 1922, and 4,067 in the following year. A survey in September, 1923, showed improvement in 55·9 per cent., while in 36 per cent. more the progress of the disease had been checked, 6·4 per cent. were worse, and 1·7 per cent. had died, and concluded that under the circumstances 'it is felt that this result is far from discouraging.' This opinion is strengthened by the following figures which speak for themselves, showing the percentages improved after different periods of treatment.

| | | | | | |
|---|---|---|---|---|---|
| Months of Treatment ... | Under 3 | 3–6 | 6–9 | 9–12 | 12–15 |
| Percentage Improved ... | 26 % | 42 % | 74 % | 81 % | 93 % |

In Hawaii still more instructive figures are now available on account of the much longer duration of the treatment by ethyl chaulmoograte at the Kahili hospital near Honolulu, where all newly discovered lepers are first sent for diagnosis and treatment, and the most advanced unamenable ones eventually drafted on to the Molokai settlement. From 1912 to 1918, before the introduction of the new improved treatment, the yearly discharges on parole as recovered averaged 6·5, during the first two fiscal years ending on June 30th, 1919, and 1920, of the new treatment, 20 and 31, respectively, were thus discharged, in 1920-21 there were 115 new admissions and no less than 94 discharged recovered; of these 23 relapsed, so in 1921-22 under stricter rules only 26 were paroled, the number rising again the following year, 1922-23, to 52. During the last three years, when the full effects of the improved treatment were obtained, there were 310 admissions and 172 discharged recovered, or 55·5 per cent. of the number admitted during the same period, all after examination by a board of three experienced doctors; 16 died of complicating diseases, mostly from tuberculosis. During the last four years 92 cases were transferred to Molokai as not yielding to treatment, a yearly average of 22·3 per cent., or less than one-fourth of the average yearly admissions. Further, of a total

of 249 paroles in the ten years, 1912-21, 31, or 12·4 per cent. relapsed, some of whom cleared up again on further treatment.

To understand the full significance of these figures we must recall that the usual annual mortality among the advanced cases seen in leper asylums and settlements is rarely less than 10 per cent., and is often higher ; the recent Hawaii admissions of the last three years show a diminution each year of about 10 per cent. on the numbers of the previous year, which it is not unreasonable to hope may continue, in view of the numbers of cases now being cleared up in the early stages before they have had the prolonged opportunities of infecting their households, as when they hid their disease in the absence of any efficient treatment, instead of declaring it voluntarily as so many have done recently. If such proves to be the case I estimate that the total known Hawaii lepers will decline by over 40 per cent. within one decade, or far more rapidly than has ever been known in a tropical country in the absence of any effective treatment : clearly illustrating the value of the new methods in the age-long struggle to stamp out leprosy.

The immense importance of treating leprosy in its early stages is proved by the following Honolulu figures of the percentages of recovered cases in relation to the duration of the disease on commencing treatment.

| Years Duration... | Under ½ | ½–1 | 1–2 | 2–3 | 3–4 | 4–5 | 5–8 | 8–10 | Over 10 |
|---|---|---|---|---|---|---|---|---|---|
| Recovered ... | 44% | 18·5% | 17% | 10·5% | 9% | 4% | 4% | 3% | 9·5% |

The much higher recovery rate in early cases is well shown by these figures, while in my own cases I found that 50 per cent. of cases treated within three years of the onset cleared up completely, but only 25 per cent. of those from three to fifteen years' duration did so. It is also of great significance that in Hawaii in former days the discharge as recovered scarcely ever occurred in the nodular type, while under the new treatment just over two-thirds of those released on parole were of this previously incurable form. The younger age groups also yielded the largest proportion of successes.

These results are also in accordance with Muir's recent statement : 'We have found, further, that most early cases lose all signs of active disease within a few months. . . . The most hopeful method of dealing with leprosy must, therefore, depend on early diagnosis and treatment.' Dr. Travers, who is now in charge of a leper asylum

in the Malay States after long service there, has recorded his opinion that 'if taken in time, the progress of the disease can be arrested and that in a large proportion of cases, leprosy can be actually cured.' Muir also states 'Our experience shows that leprosy can almost always be diagnosed long before it becomes infectious; that is to say, the disease may be recognised by clinical signs long before bacteriological examinations are positive,' and he rightly, in my opinion, advocates the multiplication of leprosy clinics, something on the lines of tuberculosis dispensaries in Britain, but with far brighter prospects of successfully eradicating the plague.

*Can Lasting Cures be Obtained?* It is now established that most early and some more advanced cases lose all symptoms and infectivity of leprosy under treatment, but it is still too early to say in what proportion of such cases the whole of the lepra bacilli have been destroyed with consequent permanent cure apart from reinfections. The frequency with which relapses occur in tuberculosis after apparent cure by sanatorium treatment necessitates the greatest caution in claiming permanent results in leprosy before sufficient time has elapsed to justify these claims. Nevertheless, the outlook even in this respect appears to me to be far more favourable than in tuberculosis, for one of my early patients has now remained free for over eight years from all signs of the disease, except for the crippling of one hand due to irreparable damage to an ulnar nerve, although he has had no treatment during the last three years; several more cases had remained free from all symptoms for five and six years when I last heard from them. In Hawaii 88 per cent. of the paroled cases have remained well for several years, and although it is advisable to continue some treatment for a year or so after apparent recovery, yet with this precaution there are now good grounds for hoping that the results will be permanent in a large proportion of the earlier cases at any rate. Moreover, the evidence that the treatment actually leads to destruction of the lepra bacillus in the body not only places it in a more hopeful position than the building up of the resisting powers of the tissues by the sanatorium treatment of tuberculosis, but also raises the still more important question of the possibility of applying the new line of treatment to the white man's scourge, tuberculosis.

# RAVAGES CAUSÉS PAR LA MOUCHE DE GOLOUBATZ EN ROUMANIE ; SES ATTAQUES CONTRE LES ANIMAUX ET CONTRE L'HOMME

PAR

T. CIUREA

PROFESSEUR DE PARASITOLOGIE À LA FACULTÉ DE MÉDECINE VÉTÉRINAIRE DE BUCAREST

ET

DR. G. DINULESCU,

VÉTÉRINAIRE CAPITAINE DE L'ARMÉE ROUMAINE

PLATES XVIII-XX

## I. LES ATTAQUES CONTRE LES ANIMAUX

INTRODUCTION. En Europe, un des plus grands foyers de Simulies est celui représenté par la mouche de Goloubatz (*Simulium columbaczense* Schiner)*, dont l'origine se trouve en Jugoslavie dans les départements de Pojarevatz et Craina du côté droit du Danube et en Roumanie dans les départements du Banat : Timis-Torontal et Caras-Severin du côté gauche. Dans cette région montagneuse, qui mesure environ 22000 Km. il n'existe guère de petits cours d'eau, où l'on ne puisse trouver l'une des formes évolutives de cette Simulie (Tömösváry).

De ce foyer à peu près chaque année au printemps,† la mouche de Goloubatz fait ses invasions en essaims plus ou moins grands dans quelques-uns de nos départements de Transylvanie et d'Olténie. La population rurale de ces départements connait assez bien la mouche et plus ou moins aussi les moyens pratiques d'en protéger le

* *Simulium columbaczense* a pris son nom de l'ancien château de Goloubatz situé sur la rive droite du Danube, dans le département de Pojarevatz en Jugoslavie ; on croyait autrefois, que cette Simulie faisait son évolution principalement dans les petits ruisseaux, qui coulent dans le voisinage de ce château.

D'après les recherches que nous avons entreprises sur les Simulies de la faune de Roumanie, il s'agit ici, non seulement du *Simulium columbaczense*, mais aussi d'autres espèces de *Simulium*, dont les nymphes possédant 4, 6 et 8 trachées de chaque côté, se trouvent en grand nombre dans les petits ruisseaux de cette région. Nous espérons pouvoir revenir sur cette question lorsque notre étude sera terminée.

† Dans le Banat d'après une légende locale, les mouches de Goloubatz s'envolent au printemps d'une grotte nommée „ Gaura cu musca " (la grotte à la mouche) qui se trouve sur la rive gauche du Danube, dans les montagnes du département de Caras-Severin, près du village de Coronini. D'après cette légende les mouches prennent naissance ici de la tête putréfiée d'un dragon tué par un Hercule.

bétail, de sorte que le nombre d'animaux tués annuellement par cette Simulie, ne dépasse que rarement quelques dizaines de victimes. Mais exceptionnellement cette année (1923), d'immenses essaims de Simulies ayant l'apparence de nuages, ont invadé non seulement le Banat, la Transylvanie et l'Olténie (à l'exception du département de Romanati), mais aussi une grande partie de la Valachie, où la population ne connaissait pas la mouche de Goloubatz et les moyens d'en protéger les animaux. C'est ainsi qu'on peut expliquer qu'en quelques jours plus de 16000 animaux ont été tués par les piqûres vénimeuses de cet insecte. La mouche a tué aussi de nombreux animaux sauvages tels que chevreuils, lièvres et renards. Les hommes même eurent à en souffrir.

*Les deux essaims de Simulies ; le temps de leur apparition ; la direction qu'ils ont suivie ; les départements qu'ils ont invadé et la durée de leur invasion.* D'après les informations fournies par les Médecins Vétérinaires, les mouches de Goloubatz ont fait leur apparition en petit nombre dans le département de Caras-Severin du Banat. Vers le milieu du même mois elles sont devenues plus nombreuses et ont formé deux grands essaims, dont l'un s'est dirigé tout d'abord vers le Nord et l'autre un peu plus tard vers l'Est.

(*a*) *Le premier essaim,* dirigé vers le Nord, a invadé les départements suivants du Sud-Ouest du Banat et de la Transylvanie :

Le 15 Avril les départements de Timis-Torontal et de Hunedoara, le 26 Avril le département de l'Arad, le 3 Mai le département de Bihor, le 8 Mai le département d'Alba de jos et le 10 Mai le département de Turda-Aries. La progression des mouches s'est arrêtée ici.

Dans le Nord, l'invasion de cette région dura environ jusqu'aux derniers jours du mois de Mai. Dans le Sud, spécialement dans le département de Caras-Severin, elle a été de plus longue durée, —pendant tout le mois de Juin. Les autorités de la circonscription de Moldova-Nouä ont rapporté que l'invasion s'est prolongée ici jusqu'à la fin du mois de Juillet, mais la commission chargée de l'étude de ces insectes n'a pas pu les observer même dans les premiers jours de ce mois.

(*b*) *Le second essaim* de mouches de Goloubatz, en partant aussi du département de Caras-Severin ou des régions voisines s'est dirigé vers l'Est, un peu plus tard que celui du Nord et a invadé d'abord huit départements de l'Olténie et de la Valachie (spécialement

ceux qui sont situés le long du versant méridional des Carpathes) dans l'ordre suivant :

Le 21 Avril le département de Mehedinti, le 23 Avril les départements de Doljet de Gorj, le 25 Avril le département de Vâlcea. Le 28 Avril elles sont passées en Valachie dans les départements de l'Olt et l'Arges, le 30 Avril dans le département de Muscel et le 2 Mai dans le Nord du département de Dâmbovita. L'invasion fut arrêtée ici par une pluie, qui a fait disparaître les mouches.

Ensuite par les défilés des Carpathes de l'Olténie et de la Valachie, les mouches de Goloubatz ont invadé trois départements de la Transylvanie situés sur le versant septentrional de ces montagnes. Le 23 Avril elles sont passées du département de Gorj dans celui de l'Hunedoara ; le 4 Mai les mouches du département de Vâlcea ont invadé le département de Sibiu et le 5 Mai les Simulies de Muscel ont passées dans le département de Fagaras.

L'invasion a duré dans ces départements jusqu'aux derniers jours du mois de Mai, c'est à dire plus de 40 jours.

En résumé on peut dire que ces Simulies ont invadé 17 départements de la Roumanie, dont 2 appartiennent au Banat, 7 à la Transylvanie, 4 à l'Olténie et 4 à la Valachie, ce qui représente environ un huitième de la surface totale du pays.*

*Les mouches de Goloubatz ont été apporté par le vent.* Il y a deux opinions en ce qui concerne le mouvement des essaims de Simulies : d'après l'une, les essaims se déplacent en remontant le long des grands cours d'eaux ; d'après l'autre, ils sont transportés passivement par le vent. Si nous observons sur la charte d'invasion ci-joint la direction suivie par les Simulies, nous voyons qu'elle est perpendiculaire sur celle des cours d'eaux. Ce fait démontre qu'il s'agit ici d'un transport passif de ces insectes. D'ailleurs à l'occasion de l'enquête que nous avons faite parmi les Médecins Vétérinaires relativement aux différentes questions se rapportant à l'invasion,

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* En même temps que la Roumanie, la Jugoslavie et la Bulgarie furent envahies par les Simulies, mais le nombre d'animaux tués dans ces deux derniers pays fut beaucoup moins que celui de la Roumanie.

D'après les rapports officiels de la Jugoslavie, la mouche a invadé sept départements du Sud-Est du pays (Vrania, Morava, Pojarevatz, Pirot, Krusevatz, Timoc et Toplic), où elle a tué 1552 animaux, dont 25 chevaux, 1 âne, 910 bovins, 251 moutons, 90 chèvres et 295 porcs.

En Bulgarie furent attaqués trois départements du Nord-Ouest (Widin, Vrata et Sophia). Dans le premier département la mouche de Goloubatz a tué 1500 animaux, dont 42 chevaux, 4 ânes, 503 bovins, 264 buffles, 306 moutons, 183 chèvres et 199 porcs. Dans les deux autres départements aussi la mouche a tué un grand nombre d'animaux.

TABLEAU I

Animaux tués par la mouche de Goloubatz pendant l'invasion de l'année 1923.

| Provinces | No. courants | Départements | No. des communes invadées | Chevaux | Ânes | Bovins | Buffles | Moutons | Chèvres | Porcs | Total par départements | Total par |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BANAT ... ... | 1 | Caras-Severin ... | 42 | 5 | 1 | 42 | — | — | 2 | 4 | 54 | 1 |
| | 2 | Timis-Torontal ... | 20 | 210 | — | 1221 | 15 | — | — | — | 1446 | |
| TRANSYLVANIE | 3 | Hunedoara ... ... | 189 | 22 | 1 | 269 | — | 9 | 20 | 19 | 340 | |
| | 4 | Arad ... ... ... | 35 | 2 | — | 77 | — | 9 | 37 | 11 | 136 | |
| | 5 | Bihor ... ... | 26 | 1 | — | 53 | — | 1 | 10 | 5 | 70 | |
| | 6 | Alba de Jos... ... | 10 | 1 | — | 41 | — | — | — | — | 42 | |
| | 7 | Turda-Aries... ... | 9 | — | — | 5 | — | — | — | — | 5 | |
| | 8 | Sibiu ... ... | 13 | 5 | — | 57 | — | — | — | 2 | 64 | |
| | 9 | Fagaras ... ... | 2 | — | — | 2 | — | — | — | — | 2 | |
| OLTÉNIE ... | 10 | Mehedinti ... ... | 145 | 216 | — | 1055 | — | 106 | 13 | 102 | 1492 | |
| | 11 | Dolj ... ... ... | 82 | 312 | 20 | 1249 | — | 60 | — | 175 | 1816 | |
| | 12 | Gorj ... ... ... | 114 | 107 | 11 | 560 | — | 281 | 84 | 520 | 1563 | |
| | 13 | Vâlcea ... ... | 111 | 391 | 10 | 1756 | 12 | 114 | 120 | 940 | 3307 | |
| VALACHIE ... | 14 | Arges ... ... | 75 | 184 | 6 | 2884 | — | 298 | 113 | 952 | 4437 | |
| | 15 | Muscel ... ... | 54 | 121 | 11 | 1027 | 1 | 37 | 24 | 117 | 1338 | |
| | 16 | Olt ... ... ... | 9 | 4 | — | 106 | — | — | — | 12 | 122 | |
| | 17 | Dâmbovita ... ... | 9 | 4 | — | 188 | — | — | 37 | 11 | 240 | |
| | | Total général ... | 945 | 1585 | 60 | 10,592 | 28 | 915 | 460 | 2,834 | 16,474 | |

presque tous nous ont fait savoir que les mouches ont été apportées de la direction de leurs lieux d'origine par le vent, qui a soufflé quelque temps avant l'invasion.

En dehors de ce mode de transport des Simulies à de grandes distances, nous croyons, comme d'autres auteurs, que ces insectes peuvent se déplacer à des distances moins longues, d'une localité à l'autre en accompagnant les animaux qu'ils désirent attaquer. Ainsi on a vu fréquemment les villages envahis le soir par des Simulies venant avec les animaux qui rentraient des pâturages.

*Le nombre d'animaux tués, leur valeur marchande et l'indemnisation accordée par le gouvernement roumain aux fermiers les plus éprouvés.*

D'après les dates, qui nous ont été fournies par le Ministère de l'Agriculture, les mouches de Goloubatz ont tué pendant l'invasion 16,474 animaux domestiques, dont la répartition par espèces, provinces et départements se trouve dans le Tableau I ci-joint.

En consultant ce tableau nous voyons, que ce sont les bovins qui ont payé le plus grand tribut à la mort (10,592), après viennent les chevaux (1,585) et les porcs (2,834), ensuite les moutons (915) et les chèvres (460).

En ce qui concerne la gravité des pertes, les départements peuvent être rangés dans l'ordre suivant : En première ligne les départements de l'Arges, de Vâlcea, de Muscel et une petite portion du département de l'Olt et de Dâmbovita. En deuxième ligne viennent les départements de Mehedinti, de Dolj, de Gorj et une partie du département de Timis-Torontal. En troisième ligne sont : la moitié du Nord du département de Caras-Severin, le département de l'Hunedoara et les parties invadées des départements de Alba de Jos, de Turda-Aries, de l'Arad, de Bihor, de Sibiu et de Fagaras. Enfin en quatrième ligne vient la moitié méridionale du département de Caras-Severin (voir la charte).

En général on peut dire que le plus grand nombre d'animaux tués s'est trouvé dans les départements de l'Olténie (8,178) et de la Valachie (6,137), tandis que dans les départements du Banat ce nombre a été seulement de 1,500 et dans ceux de la Transylvanie encore plus réduit (659).

Il est intéressant de mentionner que beaucoup de Médecins Vétérinaires, ont rapporté que les piqûres des mouches de Goloubatz ont été mortelles seulement pendant les premiers quatre ou cinq

CHARTE DE LA ROUMANIE ; LES DÉPARTEMENTS INVADÉS PAR LA MOUCHE DE GOLOUBATZ AU PRINTEMPS DE L'ANNÉE 1923.

jours de l'invasion, quoiqu'elles soient restées plus de deux ou trois semaines dans une même localité et que leurs piqûres fussent aussi nombreuses qu'au commencement de l'invasion. Cela s'explique, nous croyons, par l'établissement d'un état d'immunité chez les animaux qui ont resisté à une première attaque. Cette hypothèse est corroborée d'ailleurs par le fait que la mortalité la plus réduite a été constatée parmi les animaux de la moitié inférieure du département de Caras-Severin, c'est à dire là, où la mouche sévit d'une manière endémique. À cela s'ajoute certainement le fait que la population de cet endroit est mieux renseignée sur les moyens de prévention contre l'attaque de l'insecte.

Dans le Tableau II ci-joint nous avons calculé le pourcentage des pertes dans les départements les plus ravagés seulement.

TABLEAU II

Le pourcentage d'animaux tués par la mouche de Goloubatz dans quelques départements de la Roumanie

| | Départements | Chevaux Pertes % | Bovins Pertes % | Porcs Pertes % | Moutons Pertes % |
|---|---|---|---|---|---|
| 1 | Arges ... ... ... | 1.90 | 3.22 | 1.65 | 0.17 |
| 2 | Vâlcea ... ... ... | 4.63 | 1.86 | 2.19 | 0.07 |
| 3 | Muscel ... ... ... | 2.10 | 1.62 | 0.45 | 0.02 |
| 4 | Mehedinti ... ... ... | 1.13 | 0.82 | 0.14 | 0.04 |
| 5 | Dolj ... ... ... ... | 0.79 | 0.80 | 0.15 | 0.02 |
| 6 | Gorj ... ... ... ... | 1.75 | 0.49 | 1.01 | 0,18 |
| 7 | Timis-Torontal ... ... | 0.21 | 0.95 | — | -- |

Ainsi on voit par exemple dans ce tableau que le département d'Arges a perdu 3·22% de ses bovins et le département de Vâlcea a perdu 4·62% de ses chevaux et 2·49% de ses porcs.

Enfin dans le Tableau III ci-annexé nous avons donné d'après les évaluations officielles la valeur en lei des animaux tués.

Nous voyons de ce tableau que la valeur des animaux tués par la mouche de Goloubatz représente un capital d'environ 63,059,000 lei,* auquel il faut encore ajouter les dommages causés par la cessation du travail agricole, qui en est résultée.

* 800 lei (*c.*) = £1 0 0.

Le gouvernement roumain, désirant venir en aide aux fermiers les plus eprouvés, a mis à leur disposition une somme de 15,000,000 lei pour refaire leur bétail.

*La manière dont la mouche de Goloubatz attaque les animaux et le moment de la journée où elle est agressive.* A leur arrivée les Simulies se posent sur le corps des animaux, spécialement sur les muqueuses des orifices naturels et sur les parties les plus fines de la peau : lèvres, narines, yeux, oreilles, fanon, poitrine, face ventrale de l'abdomen, mamelles, organes génitaux, anus et la face interne des

Tableau III

La valeur en lei des animaux tués

| Espèce animale | No. d'animaux tués | Valeur en lei |
|---|---|---|
| Chevaux ... ... ... ... ... ... ... | 1,585 | 6,340,000 |
| Ânes... ... ... ... ... ... ... ... | 60 | 240,000 |
| Bovins ... ... ... ... ... ... ... | 10,592 | 52,960,000 |
| Buffles ... ... ... ... ... ... ... | 28 | 135,000 |
| Moutons ... ... ... ... ... ... ... | 915 | 366,000 |
| Chèvres ... ... ... ... ... ... ... | 460 | 184,000 |
| Porcs ... ... ... ... ... ... ... | 2,834 | 2,834,000 |
| Total ... ... ... ... ... ... | 16,474 | 63,059,000 |

membres. Les mouches de Goloubatz enfoncent assez profondément leur trompe dans la peau et y restent bien fixées jusqu'à ce qu'elles soient bien gorgées de sang, environ cinq minutes. Quelquefois elles deviennent tellement grosses qu'elles ne peuvent plus s'envoler.

Le plus souvent les bestiaux attaqués au pâturage s'agitent et s'enfuient à des dizaines de kilomètres. Quelquefois ils entrent dans l'eau pour se débarrasser des mouches. Les chevaux et les porcs rentrent dans leurs écuries. Les moutons et les chèvres ont moins à souffrir s'ils ne sont pas tondus. Nous avons observé aussi que les animaux à robe noire sont plus attaqués que les autres. Ainsi s'explique, je crois, l'affirmation de Tömösváry, que parmi nos animaux domestiques ce sont les buffles qui ont le plus à souffrir

pendant l'invasion de la mouche. Cet auteur a trouvé l'explication de ce fait en ce que les buffles étant des animaux indolents ne peuvent pas se défendre contre les mouches.

Presque dans tous les départements les mouches sont restées peu de temps dans la plaine, et se sont refugiées bientôt dans les forêts. Là, à l'abri des intempéries, elles ont pu vivre longtemps en faisant un grand nombre de victimes parmi les animaux.

En ce qui concerne le moment où les mouches sont agressives, nous avons observé qu'elles attaquent seulement pendant la journée, depuis le lever du soleil jusqu'à 10 heures du matin et dans l'après midi environ de 4 heures jusqu'au soir.

Pendant les heures les plus chaudes de la journée et pendant la nuit, les Simulies restent cachées dans des cavernes ou sous les feuilles des plantes.

*Symptômes locaux.* La piqûre des Simulies ressemble à celle de la puce. Le sang en suinte et quand plusieurs piqûres se trouvent dans la même région, celle-ci ressemble à une vaste plaie saignante. La peau d'un animal attaqué ainsi prend un aspect tacheté par les nombreuses piqûres, dont la plus grande partie confluent, formant des taches hémorragiques plus ou moins grandes. A l'endroit piqué se produit une induration sous la forme d'un petit bouton. Dans les cas des morsures multiples, les boutons confluent dans un grand oedème inflammatoire, dur et très douloureux, ce qui fait que les animaux se soustraient à la palpation. L'oedème de la tête chez le cheval donne l'impression d'un animal atteint de la Fièvre pétéchiale. L'oedème de l'abdomen, du fourreau et du pénis s'observe fréquemment chez le cheval, tandis que celui du fanon est commun chez le boeuf. L'oedème abdominal est quelquefois si grand qu'il donne l'impression d'un animal avec une hernie ventrale. Celui du pénis empêche la miction et cause la mort par l'urémie. Les animaux avec des oedèmes aux membres ont la marche difficile comme ceux atteints d'un rhumatisme articulaire.

*Symptômes généraux.* Les animaux gravement piqués manifestent les symptômes d'une asphyxie imminente : ils se meuvent très difficilement, ont la bouche entrouverte, la langue pendante, les yeux anxieux et la respiration suffocante ; enfin ils tombent et meurent en quelques heures (Triminoiu). Cela s'observe le plus souvent chez les bêtes surprises au travail par la mouche et qui

n'ont pas la possibilité de se défendre. Les animaux ayant souffert à cause des piqûres de la mouche, mais qui ne succombent pas dans les premières heures après l'attaque deviennent très irritables, ne mangent plus, et ont des frissons. La température est normale ou très peu élevée, la respiration accélérée et dispnéique, le pouls accéléré; on observe assez fréquemment un pouls veineux très manifeste. Les animaux deviennent apatiques, la respiration et le pouls sont presque imperceptible. La température descend sous la normale. Les victimes restent à peu près tout le temps couchés en découbitus sterno-abdominal (la tête tournée vers la queue comme les vaches atteintes de la Fièvre vitulaire (Velcov) ). Ils conservent tranquillement cette attitude jusqu'à la mort qui survient après 6 ou 7 jours.

L'état des animaux chez lesquels la maladie évolue vers la guérison s'amende graduellement et revient à la normale après 7 ou 8 jours.

*Lésions.* A l'autopsie des animaux morts à la suite des piqûres de cette Simulie on a constaté des congestions et des dégénérescences des principaux viscères, spécialement du coeur, du foie et des reins.

*Moyens pratiques pour prévenir à l'attaque des animaux.* Dans les départements les plus souvent invadés, les moyens employés par la population rurale contre l'attaque de cet insecte sont les suivants :—

À l'arrivée de la mouche tous les animaux sont retenus pendant la journée dans des écuries sombres et on les conduit au pâturage et au travail seulement pendant la nuit. Pour empêcher la mouche d'entrer dans les écuries, on produit de la fumée en brulant du fumier dans le voisinage de la porte.

Si l'on est obligé de conduire les animaux au pâturage pendant la journée on produit autour d'eux de la fumée. De même on protège les animaux qui travaillent, en brûlant de la paille et du fumier dans un seau que l'on suspend à l'extrémité antérieur du timon.

Les fermiers préviennent encore à l'attaque de la mouche par des onctions faites sur les parties les plus vulnérables du corps de différentes décoctions et mixtures. Parmi les décoctions on emploie celles des feuilles d'absinthe, de noyer, de noisetier et même de tabac dans de l'eau ou du vinaigre (en proportion moyenne 1/10). On emploie aussi des mixtures de goudron minéral ou végétal avec

de l'axonge rance dans des proportions égales, ou même de l'axonge seule.

Il est intéressant de savoir comment les paysans de nos montagnes, spécialement ceux du département de Mehedinti, fabriquent le goudron végétal en utilisant le bois de *Pinus silvestris*. Dans ce but on fait dans la terre une fosse conique d'une profondeur d'un mètre, avec la base en haut, dont le fond communique avec l'extérieur par l'intermédiaire d'un petit canal (voir la figure du texte No. 1). Dans cette fosse on met des morceaux de bois de pin coupés en longueur, longs de 1 m. et larges de 4-5 cm. On range le bois dans la fosse obliquement, de sorte qu'il forme un cône qui dépasse la fosse d'un demi mètre. On couvre le bois qui reste en dehors avec de la terre en y laissant une petite ouverture à la partie supérieure par où on allume le bois. La combustion se fait ainsi de haut en bas.

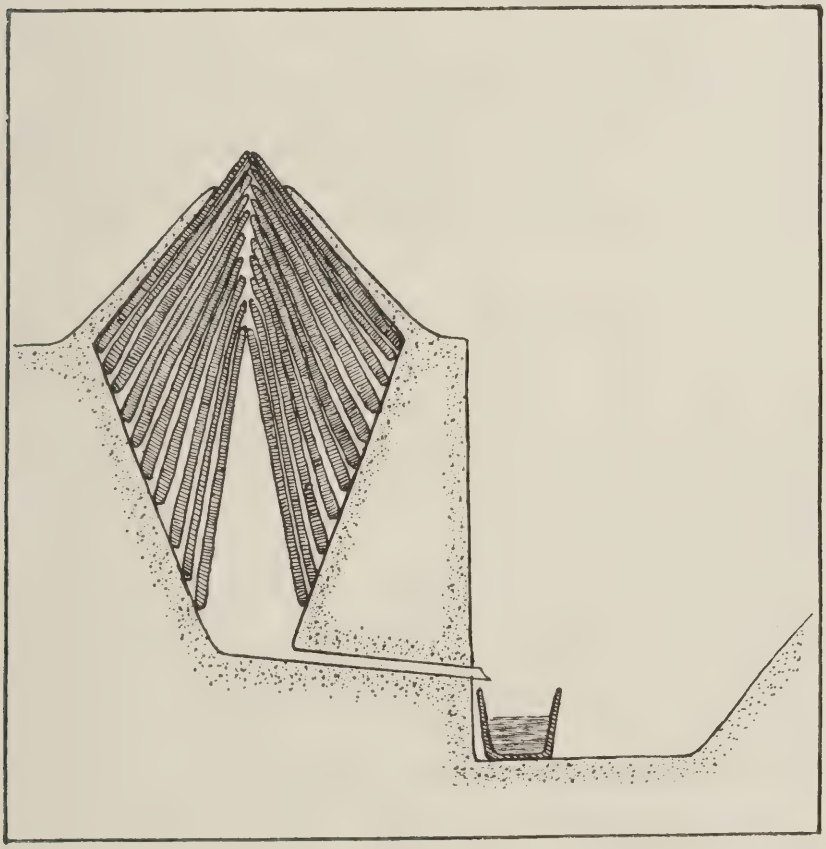

Fig. 1

La résine qui en distille, s'écoule par le canal inférieur de la fosse dans un vase. On mélange cette résine avant de l'employer, avec du lait et de la farine, en formant une pâte bien adhérente aux poils. Avec cette pâte on fait d'abord des onctions générales et on les répète de trois jours en trois jours sur les parties les plus fines et les plus vulnérables du corps.

*Moyens curatifs.* En ce qui concerne les moyens employés par nos fermiers pour guérir les animaux piqués par la mouche de Goloubatz il faut citer : Les frictions ou les compresses faites sur les régions malades avec de la saumure ordinaire ou de la saumure préparée avec une solution de vinaigre.

Les Médecins Vétérinaires ont employé des lotions ammoniacales pour neutraliser l'action du venin inoculé dans les plaies. De même on a recommandé des bains froids ou l'application de la glace pour combattre l'inflammation locale.

Comme médication interne on a employé avec beaucoup de succès les toniques cardiaques suivants : Infusions de café, de feuilles de digitales et injections de caféine. On pratique aussi des saignées.

Enfin, d'après les affirmations de nos Médecins Vétérinaires beaucoup d'animaux gravement piqués par cet insecte ont été abattus par les propriétaires et leur viande a été consommée sans aucun danger.

## II. L'ATTAQUE CONTRE L'HOMME

L'homme aussi eut à souffrir cette année, à la suite de la grande invasion de la mouche de Goloubatz en Roumanie.

D'après les informations données par les Médicins les piqûres de ces insectes ont provoqué chez l'homme des manifestations morbides locales et générales, mais non des accidents mortels comme chez les animaux.

C'est à Monsieur le Docteur Cădere (Médecin de la ville de Câmpulung, département de Muscel) que nous devons une très intéressante étude clinique sur les accidents produits chez l'homme par les piqûres de cet insecte.* En voici un résume :

*Symptômes locaux.* La mouche pique l'homme à la tête, aux mains et aux pieds. Peu de temps après la piqûre, on a la sensation

* Des observations semblables ont été faites par nous cette année chez les paysans du département de Gorj, piqués par la mouche de Goloubatz (Photographies 6 et 7).

de cuisson et un peu plus tard la sensation d'une brûlure intense. De la plaie, qui est très fine s'écoule un véritable flot de sang, qui est en disproportion avec sa petitesse. Ce sang a perdu ses caractères normaux ; il est plus fluide et se coagule avec beaucoup de difficulté.

Quelques heures plus tard apparait autour de la piqûre une tache rougeâtre presque circulaire semblable à celle produite par la puce ou la punaise.

Après 12-24 heures ces taches s'aggrandissent et lorsqu'elles sont nombreuses s'unissent et forment des plaques d'un aspect erysipélateux. Ces plaques aux bords un peu élevés, d'une couleur de framboise, peuvent occuper des régions entières : face, bras, jambes. Celles-ci semblent alors atteintes d'une véritable lymphangite réticulaire. La région malade est plus sensible et plus chaude (37°5-37°8).

Aux endroits piqués il se forme de petits boutons, du sommet desquels s'écoule une sérosité trouble.

Dans une phase plus avancée et dans les cas plus graves, les lésions évoluent vers une infiltration oedémateuse de la peau, qui devient pâle jusqu'au blanc vitreux. En palpant la région malade on provoque des douleurs très vives et on constate que l'oedème est ferme et qu'il ne garde pas les emprintes des doigts, comme font les oedèmes ordinaires.

Autour des piqûres apparaissent ensuite de petites vésicules contenant une sérosité claire, disposées en demicercle ou cercle complet, semblables à celles qui entourent le centre d'une pustule maligne.*

Dans les cas légers, les vésicules se désèchent et disparaissent en 48-60 heures. Dans ceux plus graves, les vésicules se transforment en pustules, qui en 12-24 heures éclatent et donnent naissance à des ulcérations d'un aspect sale. La plaque centrale se mortifie et s'élimine sous la forme d'une escarre mesurant 2-6 cm. La régénération des tissus se fait lentement (10-15 jours) en produisant une cicatrice blanchâtre.

Les ganglions lymphatiques prennent part au procès inflammatoire dans la mesure des altérations locales. Ainsi dans les cas légers ils sont seulement sensibles, tandisque dans les cas graves ils sont hypertrophiés et très douloureux.

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* Des cas semblables ont été observés par Mr. le Dr. Drăghiescu, Médecin de la ville de Târgul Jiu.

*Symptômes généraux.* Ceux-ci varient d'après la gravité des lésions locales. Quand ces lésions sont peu importantes, on observe seulement un état d'indisposition, d'agitation et un fourmillement dans tout le corps ; quand elles sont plus graves la température baisse (36°), on constate un état d'algidité avec refroidissement des extrémités, un pouls mou, de la diarrhée et une oligurie passagère.* Au fur et à mesure que les altérations locales cèdent, les symptômes généraux disparaissent et les malades guérissent.

*Traitement.* Localement on a appliqué des onctions avec liniment ammoniacal.† Comme médication interne on a administré des diurétiques, des toniques cardiaques et du chlorure de calcium dans les cas accompagnés d'hémorragies plus abondantes.

*Prophylaxie :* Celle-ci consiste principalement dans la protection contre les piqûres de la mouche, des régions attaquables, en utilisant de différents moyens comme un masque ou un linge quelconque pour la figure, des gants et des chaussures. De même on peut prévenir à l'attaque par l'onction des régions énumérées avec des mélanges de substances grasses ou huilleuses, avec de l'ammoniaque ou des essences volatiles.

En terminant cette note nous voulons mentionner que la question de la mouche de Goloubatz a été étudiée jusqu'à présent spécialement par Schönbauer, Tömösváry et Aigner-Abafi en Hongrie ; par Moga et par Leon en Roumanie ; par Medovici et par Georgévitch en Jugoslavie ; par Dobrev et par Gheorghieff en Bulgarie.

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* D'après une communication, qui nous a été faite par Mr. le Dr. Draghiescu, les malades dans les cas plus graves sont obligés d'interrompre leur occupation pendant 8-12 jours.

† Le Dr. Draghiescu nous a informé qu'il a employé avec de bons résultats des compresses ou des lotions avec du vinaigre sur les régions malades.

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## EXPLANATION OF PLATE XVIII

FIG. 1. Les ruines du château de Goloubatz dans la Jugoslavie ; cliché pris sur la rive roumaine du Danube, à Moldova Veche, village situé vis-à-vis de ce château dans le département de Caras-Severin du Banat.

FIG. 2. La grotte " Gaura cu musca " (la grotte à la mouche) dans les montagnes du Banat, près du village de Coronini, sur la rive gauche du Danube.

Fig. 1

Fig. 2

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## EXPLANATION OF PLATE XIX

FIG. 1. Paysans roumains avec la charrue au travail du champ pendant l'invasion de la mouche de Goloubatz. On voit deux seaux attachés au timon, produisant de la fumée par la combustion du fumier.

FIG. 2. Boeuf avec l'oedème du fanon, produit par des piqûres de la mouche de Goloubatz.

Fig. 1

Fig. 2

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## EXPLANATION OF PLATE XX

FIGS. 1 and 2. Paysannes du département de Gorj, piquées par des Simulies. On voit des ecchymoses, des boutons et des oedèmes de la jambe.

FIG. 3. Peau de boeuf avec des piqûres de la mouche de Goloubatz.

Fig. 1

Fig. 2

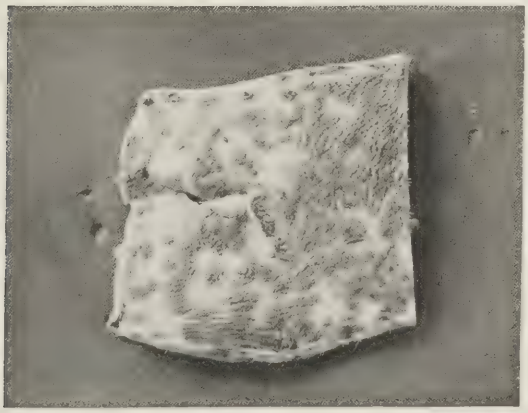

Fig. 3

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# THE SOURCE OF SOUTH AFRICAN TREMATODES

BY

F. G. CAWSTON, M.D. (Cantab.).

(*Received for publication* 4 *July*, 1924)

According to the literature that was available up till 1915, the only fresh-water snail that was held to be responsible for the development of trematode parasites of man and beast in South Africa was a minute species which had been shown to be responsible for Fasciola disease in other lands, namely *Limnaea truncatula*. I have examined some of the very few examples of this rare snail from the Transvaal ; but, as it had not been found infested with cercariae in South Africa, its existence may be regarded as of very little practical importance fron a disease-prevention point of view. The shell of *L. truncatula* is about 5 mm. in length. I have repeatedly shown that *Limnaea natalensis* is the usual carrier of the Fasciola parasites and its shell is probably the largest of its kind in the Union. I have collected an example from the Umhlangana that was fifteen-sixteenths of an inch in length. An occasional carrier of fasciolae and the usual carrier of the various schistosomes in South Africa, *Physopsis africana*, is about the same size. I found an example in the Durban suburbs that was 20 mm. in length. The shell closely resembles that of *Isidora globosa*, Morelet, of which I have obtained examples up to 19 mm. in length from Lourenço Marques. *Isidora tropica*, a common carrier of amphistomes and an occasional carrier of schistosomes, is common all over the Union. I have collected examples measuring 16 mm. in length, both from Schuttes' Draai, O.F.S., and from Durban. Another common carrier of trematodes is *Planorbis pfeifferi* and I have an example from the Umbilo 15 mm. in diameter.

We may say that the commoner fresh-water snails of South Africa that are responsible for trematode parasitic diseases, possess shells which measure from 15 to 20 mm. in length. However, *Melanoides tuberculata*, which is heavily infested at the Natal Coast, has an operculated shell which at Durban measures 31 mm. in length.

There are, however, a large number of smaller South African shells which may be overlooked in examining collections of semi-stagnant water for the possible presence of intermediary hosts. First in importance is *Isidora forskali* whose shell is about the size of *Limnaea truncatula* but dextral. In the smaller spruits all over the Union this common fresh-water snail is infested with amphistomes. These common parasites of cattle and sheep cause so few symptoms that little attention has been paid to their life-history. There is an incredible number of very minute shells to be found attached to reeds and floating wood which have escaped the attention they deserve as carriers of disease, probably because they usually reach a diameter of not more than 5 mm. and because their shells are so fragile. The largest genus of the *Ancylidae* is *Burnupia* and the shells of the various species measure from 3 to 9 mm. in length, whilst the *Ferrissia* and *Gundlachia* are seldom more than 4 mm. in length. I have repeatedly examined these snails for parasites, but could find no trace of infection, until in October, 1923, I found cercariae with divided tails in *Burnupia trapezoidea* (Bttg.) at Schuttes' Draai, O.F.S. Dr. E. C. Faust is describing these monostomes for me; they are about the same size as *Schistosoma*. Similar monostomes were present in *Burnupia capensis natalensis* from a small spruit at Escombe, Natal, in June, 1924. The head was 0·13 mm. in length and both tail and prongs 0·175 mm. in length, making a total length of this narrow cercaria of 0·48 mm. The shell from which these cercariae escaped was only 4 mm. in length. They were sporocyst-produced. In May, 1924, I found redia-produced distomes in two distinct species of *Burnupia* at Avoca, Natal. *Burnupia stenochorias* M. and P., *B. capensis natalensis*, Walker, and *B. caffra*, Krauss, are all present in this locality. The shell of one infested *Burnupia* was only 4 mm. in length and none were more than 6 mm. in length. The cercaria possessed a chain of cystogenous particles on each side of the body and measured 0·6 mm. in total length.

It is very difficult to extract the animal from these smaller shells for the examination of cercariae and, having convinced oneself of the presence of cercariae, to keep the entire shell for identification. However, I obtained numerous *Ferrissia* (?) *connollyi* from a small pool at Malvern, Natal, said to contain tortoises, and, having

extracted the animal from a shell only 3 mm. long by means of a dental needle, found it infested with *Megalodiscus sp.* (?) measuring 0·525 mm. in length and about 0·4 mm. in breadth. This pool also contained an interesting *Gundlachia* besides *Isidora forskali.*

There is a small water-lily pool in the Botanic Gardens at Durban in which are kept gold-fish and ' millions.' Attached to the leaves of the water-lilies are a few examples of *Limnaea natalensis,* the common intermediary host in this country for *Fasciola gigantica,* as well as examples of *Ferrissia natalensis* and *F. burnupi.* Microscopic examination of these *Ferrissiae* revealed, in a large proportion of snails dissected, a styletted cercaria about 0·26 mm. in total length, the undivided tail being half the length of the body. One infected example measured only 2 millimetres in length. The identity of this distome is being investigated ; but its presence in so minute a shell emphasises the importance of examining shells so minute as those of *Ferrissia* and *Gundlachia* for the possible presence of trematode parasites. The *Limnaeae* were free from infestation.

Various cercariae have been obtained from *Planorbinae* no more than 4 mm. in diameter and *Segmentina planodiscus,* whose shell is only slightly larger, harbours a rather large eye-spotted schistosome at the Durban Country Club.

South African trematodes develop more frequently in fresh-water snails whose shells are from 15 to 20 mm. in length ; but those species whose shells are no more than 4 to 6 mm. in diameter are occasional carriers of these parasites and prophylactic measures must be directed towards the possible eradication of all varieties, now that, for the first time, trematodes have been isolated from *Ferrissia* and from more than one species of *Burnupia.* In a young country there is a tendency to overlook the large amount of apparently unnecessary and arduous work that must be carried out before one can speak with confidence in regard to those measures which farmers must adopt, if they are to keep their farms free from parasitic diseases. Some years ago I urged the Union Government to introduce domesticated duck in order to keep down the number of Bilharzia-producing snails along the course of the Umbilo river at Pinetown and Sarnia. Instead of carrying out this suggestion, the Government investigated the desirability of introducing otters

into the river. As a matter of fact otters feed on crabs and, as crabs feed readily on snails, otters would indirectly have helped to increase the number of fresh-water snails in this badly-infested river.

Careful study of Bilharzia infection along natural history lines in Natal, over a period of several years, confirms the opinion of other workers and convinces me that our efforts must be directed towards :—

(1) The introduction of domesticated duck wherever possible.

(2) The use of lime or of Ross's larvicide in small collections of water.

(3) Drying up collections of water, at any rate for a week at a time, and this would appear to be the best method of dealing with the minuter fresh-water snails.

(4) Though it has only a limited application, at seaside resorts, in Natal we might follow nature's example where the inflow of sea-water into the lagoons destroys those fresh-water snails that are continually being carried down to the lagoons.

(5) The collection and destruction of such species as *Melanoides tuberculata*, whose operculated shell is to be found in incredible numbers in the Durban water-cress beds, and has been shown to be too stout for ducks to eat.

# LOCOMOTOR ATAXIA WITH CHARCOT'S JOINT DISEASE IN A NEGRO

BY

J. L. PAWAN, M.B., Ch.B. (Edin.), D.P.H. (Lon.),

GOVERNMENT BACTERIOLOGIST, TRINIDAD, B.W.I.

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PLATE XXI

*History.* Patient a negro, 58 years of age, was born in Trinidad, and has lived there all his life. When 19 years old he developed a chancre on his penis which healed readily with local application. Seven years later he married ; a year after marriage his first child was born. His wife gave birth to eleven children all of whom died either a few days or a few months after birth except the fifth and seventh who are alive and well.

He was apparently in good health up to twelve years ago, *i.e.*, twenty-seven years after the appearance of the chancre, when he began to suffer from (1) periodic attacks of sharp shooting pains about his knees and legs with a tendency to fall when walking ; (2) hot burning sensations about his body ; (3) difficulty of micturition ; (4) tenderness and pains about his knee-joints which gradually increased in size. The pains about his knees persisted for two years and the difficulty with micturition for six years. He has been impotent for the last eight years.

*Clinical Signs.* A fairly well-nourished man with no evidence of mental impairment, but with characteristic knee lesions. There is relaxation of the ligaments and other soft parts of the knee-joints with enlargements of the lower end of the femur and the upper end of the tibia and loss of apposition of the contiguous surfaces of the bones, so that the upper ends of the tibiae are thrown backwards and outwards. There is some atrophy of the muscles of the legs and thighs. There is palpable thickening of the synovial membranes, but no increase in the fluid. There is an abundance of rotatory movement at the knees with flail-like movements of the

legs and a remarkable absence of pain and resistance with these movements. Flexion at the knee-joint is complete, but extension is reduced by about one quarter.

These changes are more marked on the left than on the right side.

Sensation of heat is normal, that of cold is impaired.

The knee jerk and ankle jerk are absent. There is no ankle clonus.

The Babinski is doubtful. There is incoordination of the lower extremities, but not of the upper. The hand grip is firm.

Argyll-Robertson pupil is present. Rombergism is present and well-marked. The patient's walk, though not typically ataxic, is characteristic. He rests on his stick, keeping his eyes centred on the ground and his body thrown forward, with his legs held slightly apart. The feet are brought down with a stamp, the heels touching the ground first, or the whole foot comes in contact with the ground.

The blood Wassermann by Harrison's method gave a positive reaction (++++). There is no gastric, rectal or other crisis and his only complaint is in connection with the discomfort and inconvenience due to the disorganisation of his knee-joints.

## EXPLANATION OF PLATE XXI

Locomotor Ataxia with Charcot's Joint Disease in a Negro.

Fig. 1. Anterior view.

Fig. 2. Posterior view.

Fig. 1

Fig. 2

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# THE VALUE OF URINARY EXAMINATIONS IN THE DIAGNOSIS OF MALARIA

BY

R. M. GORDON

*From The Sir Alfred Lewis Jones Research Laboratory, Freetown*

(*Received for publication* 5 *August,* 1924)

## INTRODUCTION

The work which follows was commenced in England and continued in West Africa. It was undertaken with the object of ascertaining whether the more commonly employed urinary tests and examinations have any value as aids in the diagnosis of malaria ; if they have then their importance is obvious, more especially in those only too frequent cases in which the blood is negative and the patient gives a history of having had ' fever ' a few days previously, for which he took quinine. The urines were examined for urobilinogen, urobilin, albumin, casts, indican, bile pigments, and urea percentage.

*Urobilinogen and Urobilin.* Considerable difference of opinion exists regarding the importance attachable to the presence of these pigments in the urine. Plehn (1908) believes urobilinuria to be a point of great diagnostic value, but Surveyor (1910), while working in India, was unable to confirm Plehn's observations. Acton and Knowles (1913) state

> ' Urobilin is not found in the urine of healthy persons, but is found in various fevers, *e.g.*, pneumonia, and the late stages of typhoid and Malta fevers, etc.'

In their summary they state

> ' The presence of urobilin in the urine in large quantities indicates that haemoglobin is being destroyed. The blood destruction, except in certain well-defined diseases, is due to the destruction of erythrocytes by malarial parasites. If there is no fever present the patient is in the later stages of the disease.'

Acton and Knowles made no quantitative tests and, judging by the context, the expression ' large quantities ' simply means a well-marked fluorescence with Schlesinger's solution. Simpson (1910) examined twenty-two cases of malaria and estimated the urinary urobilin quantitatively on several occasions in each individual ; he found that in simple tertian malaria the output of

urobilin is never greater than in other diseases such as pneumonia. He proceeds :

> 'In malignant tertian malaria (*P. falciparum*) a different picture is obtained ; definite urobilinuria sets in very shortly after the onset of the pyrexia, reaches its height on the second or third day and then slowly diminishes ; the normal level of excretion is reached in ten days unless a fresh paroxysm intervenes. Sometimes the increased output continues for a longer period (*e.g.*, Case 20) and in old standing cases with chronic malarial anaemia there may be a continuous raised urobilin output even in the absence of pyrexia (Case 19).'

On looking up Case 20, we find that on seven out of twenty-eight examinations the urobilin output is marked as '0,' and in Case 19, referred to in the text as 'Continuous raised urobilin output,' three out of eighteen examinations are marked '0.' Ballerstedt (1924) states in his summary that urobilin persists in the urine of convalescent malarias and for a long time after the cessation of all attacks, but is careful to point out that slight urobilinuria may be found in healthy people ; he notes that when the patient's temperature has been normal for some time the urobilinogen test is usually negative while the urobilin remains positive. Sorensen (1914) does not believe in the persistence of urobilin except for two to four days after the subsidence of fever, 'though its reappearance invariably presages a relapse.' Di Pace (1923) after the examination of some one hundred and eighty malarial urines reaches the following conclusions regarding urobilinuria : (1) It is present in all acute malarias with parasites and symptoms still present. (2) Frequent in patients who had an attack of malaria two to three months previously and in whom parasites are still present. (3) Rare in cases with a history of malaria more than three months previously clinically cured, but who show parasites after the administration of strychnine. (4) Extremely rare in latent malarias that are absolutely without symptoms. Di Pace unfortunately appears to have used no controls.

*Albumin and casts.* Nephritis as a concomitant or complication of malaria is referred to by almost all writers on malaria. Levy Moise (1923) in a paper reviewing previous workers' figures, points out its great frequency. Moise examined seventy-six cases of malaria and found albumin in 27.6 per cent. and casts in 25 per cent. Gordon (1923a) found nephritis in ten out of sixteen cases of malaria examined in England.

*Indican, bile pigments, and percentage of urea.* These will be dealt with later.

## TESTS EMPLOYED IN THE URINARY EXAMINATIONS

All urines were examined as soon after passing as possible and in hospital cases the first urine passed in the morning was always tested.

*Urobilinogen.* Two drops of Ehrlich's aldehyde reagent, which consists of a 3 per cent. solution of paradimethylaminoazobenzaldehyde in 50 per cent. hydrochloric acid, were added to five c.cs. of urine and the tube gently warmed to blood heat ; the appearance of a rose pink to scarlet colour was regarded as positive. MacCormac and Dodds (1923) when describing this reaction state ' This test when positive demonstrates the presence of a pathological amount of urobilinogen.'

In order to prevent the conversion of urobilinogen to urobilin the urine to be tested was exposed to light as little as possible ; Graham (1911) in a paper on the effects of quinine on the excretion of urinary pigments, states that he placed ten hour samples of urine in a well-lighted window for two hours in order to ' Ensure the complete conversion of urobilinogen to urobilin.' In connection with this it may be remarked that the present writer frequently noted that a urine giving a positive urobilinogen reaction was still positive after eight hours exposure to full daylight.

*Urobilin.* Schlesinger's test was used, twenty c.cs. of urine were acidulated with acetic acid and the urobilin extracted by gently inverting with five c.cs. of amyl alcohol. (The mixture must be made gently or an emulsion will result.) The amyl alcohol was then pipetted off and half a c.c. of a 10 per cent. solution of zinc chloride in absolute alcohol added to it ; the appearance of distinct fluorescence and a zinc-urobilin band in the spectrum were taken as positive ; the spectroscopic result is important, as Ballerstedt (1924) has pointed out that the blue fluorescence of quinine in the urine may influence the typical green fluorescence of urobilin.

*Albumin.* Sulphosalicylic acid was prepared by dissolving thirteen grammes of salicylic acid in twenty grammes of sulphuric acid, by warming, and after cooling, adding sixty-seven c.cs. of water (Cole (1920)). If no ring resulted on the addition of urine, albumin was regarded as absent ; it was found, especially amongst West African natives, that mucin was frequently present and gave a ring indistinguishable from albumin and it was found necessary to

exclude this by testing for increased opacity on the addition of dilute acetic acid.

*Indican.* To five c.cs. of urine in a test-tube were added one large drop of 5 per cent. potassium chlorate, then five c.cs. of strong hydrochloric acid, followed by five c.cs. of chloroform, the contents being mixed by inverting the closed test-tube a couple of times. Definite blue colouration in the separated chloroform was regarded as positive.

*Bile pigments.* Various reagents were tried and Fouchet's test, as described by MacCormac and Dodd (1923) for testing serum, was found the most satisfactory ; to a small quantity of urine was added an equal quantity of the following reagent, trichloracetic acid five grammes, water twenty c.cs. and 10 per cent. ferric chloride solution two c.cs. ; bile pigments were indicated by the appearance of a bright green precipitate.

*Urea percentage.* This was roughly estimated in the usual way with a 'Southall ureometer.'

## RESULTS

The following two points must be taken into consideration when studying the figures that follow :

(1) Amongst the cases examined in England the heading 'Malaria. Parasites present,' means that parasites were actually present in the blood at the time that the urine was passed. In the cases examined in West Africa, as it was not always possible to examine the urine on the same day as the positive blood film was obtained, all that can be said with certainty is that parasites were present within a few days previous to the urinary examination.

(2) No distinction between the different species of malaria parasites is made in the tables ; actually when compiling the figures the different urinary findings accompanying malignant tertian, benign tertian, or quartan malaria were recorded, but as the differences found were of a trivial character no record of them has been retained in the paper.

TABLE I.

Showing the frequency of urobilinogen in the urine of 200 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present |
| Number of cases examined ... | 18 | 30 | 4 | 19 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 11 | 30 | 50 | 84 | 17 | 0 | ... | 39 | 6 | 33 | 62 | 87 |

*Remarks.* In none of the cases recorded above was urobilinogen detected without urobilin also being found.

TABLE II.

Showing the frequency of urobilin in the urine of 200 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present |
| Number of cases examined ... | 18 | 30 | 4 | 19 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 11 | 53 | 75 | 95 | 39 | 10 | ... | 89 | 12 | 50 | 87 | 87 |

*Remarks.* Under the heading 'Mixed hospital cases' there are included four cases of amoebic dysentery; one of these gave a positive urobilin reaction. A case which had been operated on for amoebic liver abscess twelve months previously and which still showed signs of hepatitis was also tested, but gave a negative reaction. In connection with this it may be noted that Grall, as quoted by the *Annals of the Clin. Laboratories* (1924), 'Considers that in a colonial subject abundant urobilinuria ought forthwith to make one suspicious of amoebic hepatitis.'

TABLE III.

Showing the frequency of albumin in the urines of 202 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present |
| Number of cases examined ... | 18 | 30 | 4 | 21 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 0 | 37 | 75 | 71 | 5 | 20 | ... | 63 | 24 | 42 | 62 | 36 |

*Remarks.* The large number of albuminurias among normal natives in Sierra Leone is probably explained by the fact that the individuals examined were convicts, many of whom were known to be suffering from chronic gonorrhoea.

TABLE IV.

Showing the frequency of casts in the urine of 200 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present |
| Number of cases examined ... | 18 | 30 | 4 | 19 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 0 | 17 | 25 | 53 | 0 | 10 | ... | 25 | 3 | 29 | 12 | 12 |

*Remarks.* The low percentage of malaria cases showing casts amongst the people examined in West Africa, as compared with those in England, may be due to their having received early and efficient quinine treatment, while the cases examined in England had suffered from a lack of thorough treatment during a long sea voyage.

TABLE V.

Showing the frequency of indican in the urine of 202 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites Present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites Present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites Present |
| Number of cases examined ... | 19 | 29 | 4 | 21 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 21 | 41 | 0 | 43 | 11 | 10 | ... | 25 | 6 | 21 | 0 | 12 |

*Remarks.* Judged on these figures indicanuria would appear to be less frequent in the tropics than in England; for further European figures see Gordon (1923b).

TABLE VI.

Showing the frequency of bile pigments in the urine of 200 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites Present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites Present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites Present |
| Number of cases examined ... | 18 | 30 | 4 | 19 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 0 | 7 | 0 | 0 | 0 | 0 | ... | 0 | 0 | 0 | 0 | 0 |

*Remarks.* From this it will be seen that bile pigments were only detected in the urine of two out of 200 individuals examined; in both these cases the patient was suffering from well-marked obstructive jaundice.

TABLE VII.

Showing the frequency of urea above three per cent. in the urines of 57 individuals in England.

| | ENGLAND | | | |
|---|---|---|---|---|
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present |
| Number of cases examined ... ... ... ... | 15 | 22 | 4 | 16 |
| Percentage positive ... ... ... ... ... | 7 | 9 | 0 | 56 |

TABLE VIII.

Showing the results of urinary examinations in three cases of general paralysis of the insane, before, during, and after an attack of simple tertian malaria. (Note—' + ' signifies presence of, ' o ' absence of, and ' — ' no examination made.)

CASE I. Anophelines infected with *P. vivax* fed on patient, 31.12.23.

| | JANUARY | | | | | | | | | | | | | | | | | FEBRUARY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date, 1924 ... ... | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 16 | 18 | 19 | 21 | 23 | 25 | 27 | 28 | 29 | 31 | 2 | 4 | 6 | 8 | 10 and 12 |
| First temperature ... | + | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Parasites ... ... | o | o | + | + | + | + | + | + | + | + | + | + | + | + | + | + | o 30=o | o | o | o | o | o |
| Quinine Grains 30... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + | o | o | o | o | o | o |
| Urobilinogen ... | o | o | + | + | + | + | o | o | + | + | + | + | + | + | — | + | o | + | o | o | o | o |
| Urobilin ... ... | o | o | + | + | + | + | + | + | + | + | + | + | + | + | — | + | + | + | o | o | o | o |
| Albumin ... ... | o | o | o | + | + | + | + | + | + | + | + | + | + | + | — | + | + | + | + | o | o | o |
| Casts ... ... | o | o | o | o | + | + | + | + | + | + | + | + | + | + | — | + | + | + | + | + | o | o |
| Indican ... ... | o | + | + | + | + | + | + | + | o | + | + | + | o | o | — | + | o | o | + | o | + | + |
| Bile pigments ... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | — | o | o | o | o | o | o | o |
| Urea above 3% ... | — | — | o | + | + | + | + | + | + | + | + | o | o | o | — | o | o | o | o | o | o | o |

TABLE VIII (cont.).

CASE 2. Anophelines infected with *P. vivax* fed on patient, 21.2.24.

| | February | | | March | | | | | | | | | | | | | | | | April |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ate, 1924 ... ... ... | 21 | 24 | 27 | 1 | 4 | 6 | 8 | 10 | 12 | 14 | 15 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 13 |
| irst temperature ... ... | ... | ... | ... | ... | ... | + | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| arasites ... ... ... | o | o | o | o | o 5=o | + | + | + | + | + | + | + | o | o | o | o | o | o | o | o |
| uinine grains 30 ... ... | o | o | o | o | o | o | o | o | o | o | + | + | o | o | o | o | o | o | o | o |
| robilinogen ... ... ... | o | o | o | o | o | o | o | o | o | + | − | + | + | o | o | o | o | o | o | o |
| robilin ... ... ... | o | o | o | o | o | + | o | o | + | + | − | + | + | + | o | o | o | o | o | o |
| lbumin ... ... ... | o | o | o | o | o | o | o | + | + | + | − | + | + | + | + | o | o | o | o | o |
| asts ... ... ... ... | o | o | o | o | o | o | + | + | + | + | − | + | + | + | + | o | o | o | o | o |
| idican ... ... ... | o | o | o | o | o | o | o | o | o | o | − | o | o | o | o | o | o | o | o | o |
| le pigments ... ... | o | o | o | o | o | o | o | o | o | o | − | o | o | o | o | o | o | o | o | o |
| rea above 3% ... ... | o | o | o | o | o | o | + | + | o | o | − | + | o | o | o | o | o | o | o | o |

TABLE VIII (cont.).

CASE 3. Anophelines infected with *P. vivax* fed on patient, 22.2.24. Note—Slight nephritis was present before infection.

| | February | | | March | | | | | | | | | | | | | | | | | April | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ate, 1924 ... ... ... | 22 | 25 | 28 | 2 | 5 | 7 | 8 | 9 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 10 | 18 |
| rst temperature ... ... | ... | ... | ... | ... | ... | + | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| rasites ... ... ... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | o | o | o |
| uinine grains 10 ... ... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + | + | o |
| robilinogen ... ... | o | o | o | o | o | − | + | − | + | + | o | o | + | + | + | + | + | + | + | o | o |
| robilin ... ... ... | o | o | o | o | o | − | + | − | + | + | + | + | + | + | + | + | + | + | + | o | o |
| bumin ... ... ... | Tr. | Tr. | Tr. | Tr. | Tr. | − | + | − | + | + | + | + | + | + | + | Tr. | Tr. | Tr. | Tr. | o | o |
| ists ... ... ... | + | o | o | + | o | − | + | − | + | + | o | + | + | o | o | o | o | o | o | o | o |
| dican ... ... ... | + | o | o | + | o | − | + | − | o | o | o | o | o | o | o | o | o | o | o | o | o |
| le pigments ... ... | o | o | o | o | o | − | o | − | o | o | o | o | o | o | o | o | o | o | o | o | o |
| rea above 3% ... ... | + | o | o | o | o | − | o | − | o | o | o | + | o | o | o | o | o | o | o | o | o |

*Remarks.* In Cases 1 and 2 urobilinogen and urobilin first appeared either on the same day, or else later than, the parasites; in Case 3 both urobilinogen and urobilin preceded the parasites by two days, in all three cases these substances persisted for one to five days after the blood had become negative. Albumin and casts appeared in Cases 1 and 2—and were increased in Case 3—within three days of the first positive blood finding, and persisted in Cases 1 and 2 for about a week after the disappearance of parasites; in Case 3 it will be noted that casts ceased to appear in the urine for some days prior to the blood becoming negative.

The number of days during which urobilinogen, urobilin, albumin and casts persisted in the urine after parasites had disappeared may also be noted in the following additional cases. *Case A.* Urobilinogen less than four days, urobilin more than four days, albumin more than four days, casts less than four days. *Case B.* Urobilinogen less than four days, urobilin less than four days, albumin more than four days, casts less than four days. *Case C.* Urobilinogen, urobilin, and albumin less than ten days, casts more than ten days. *Case D.* Urobilinogen and urobilin less than seven days, albumin or casts did not appear. *Case E.* Urobilinogen and urobilin more than nine days, albumin and casts less than nine days.

TABLE IX.

Showing the results of urinary examinations during pyrexial and apyrexial periods in a case of lymphadenoma. Note—The patient had never been abroad.

| | JANUARY | | | FEBRUARY | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date, 1924 ... ... ... ... ... | 29 | 30 | 31 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 17 | [illegible] |
| Temperature ... ... ... ... ... | 98 | 100 | 101 | 102 | 102 | 103 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 101 | [illegible] |
| Urobilinogen ... ... ... ... ... | — | o | — | + | — | — | o | — | o | — | + | — | + | — | o | — | [illegible] |
| Urobilin ... ... ... ... ... ... | — | + | — | + | — | — | o | — | o | — | + | — | + | — | o | — | [illegible] |
| Albumin ... ... ... ... ... | — | o | — | o | — | — | o | — | o | — | o | — | o | — | o | — | [illegible] |
| Casts ... ... ... ... ... ... | — | o | — | o | — | — | o | — | o | — | o | — | o | — | o | — | [illegible] |
| Indican ... ... ... ... ... ... | — | o | — | + | — | — | o | — | + | — | + | — | + | — | o | — | [illegible] |
| Bile pigments ... ... ... ... ... | — | o | — | o | — | — | o | — | o | — | o | — | o | — | o | — | [illegible] |
| Urea above 3% ... ... ... ... ... | — | o | — | o | — | — | o | — | o | — | o | — | o | — | o | — | [illegible] |

*Remarks.* Note the irregularity of the appearance of both the urobilinogen and the urobilin and that they do not always correspond to the pyrexial periods.

TABLE X.

Showing the frequency of a combination of urobilin, and albumin (with or without urobilinogen, casts, indican, bile pigments, or raised urea percentage), in the urine of 200 individuals in England and Sierra Leone.

| | England | | | | Freetown, Sierra Leone | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Europeans | | | | English residents | | | | Natives | | | |
| | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present | Normal | Mixed hospital cases | Clinical malaria | Malaria. Parasites present |
| Number of cases examined ... | 18 | 30 | 4 | 19 | 18 | 10 | ... | 28 | 33 | 24 | 8 | 8 |
| Percentage positive ... | 0 | 13 | 50 | 84 | 0 | 0 | ... | 46 | 0 | 25 | 62 | 37 |

## CONCLUSIONS

As regards positive findings, it can at once be stated that no individual test was found to have any real value as an aid in the diagnosis of malaria, one and all occurring with considerable frequency not only in diseases other than malaria but also in the normal individual, as is clearly shown in Tables I to VII ; so far, therefore, as any single test is concerned the results obtained are in accordance with the views expressed by Lane (1923) who remarks

> 'There emerges from all this the conclusion, old but needing as much emphasis as ever it did. In any particular case the finding of parasites is the only justification for a positive diagnosis of present malarial infection ; but the failure to find them by the ordinary techniques cannot justify a confident diagnosis that malaria is certainly absent.'

If, however, the figures furnished in the various tables are consulted, more especially Table X and the three cases recorded in Table VIII, it will be seen that a combination of urobilin and albumin in the urine, although no certain proof, is at least strong evidence in favour of malarial infection ; whether such evidence is strong enough to warrant immediate treatment with quinine is a matter which can only be decided by the exigencies of the case and the opinion of the physician.

As regards negative findings, the absence of urobilin (which implies the absence of urobilinogen ; see remarks Table I) from the urine of a suspected malaria case appears to be of very considerable value, for not less than 87 per cent. of all true cases of malaria, whether examined in England or West Africa, showed the presence of urobilin and it has been shown (Table VIII) that urobilin is sometimes a precursor of the malaria parasites and usually persists after the latter's disappearance. It is, therefore, probably legitimate to consider that a case which has had a rise of temperature within the past forty-eight hours and does not exhibit urobilin in the urine is extremely unlikely to be suffering from malaria.

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# DESCRIPTIONS OF NEW MOSQUITOS FROM SOUTH AMERICA

BY
ALWEN M. EVANS

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The material dealt with in the present paper consists largely of Culicidae collected by Dr. A. Aiken Clark on the River Amazon during his voyages to and from Manáos ; other specimens were taken or reared at Manáos by Dr. H. Wolferstan Thomas and by Dr. R. M. Gordon, and in Venezuela by Dr. M. Núñez Tovar. All of the seven species are members of the genus *Culex* and all but one belong to the sub-genus *Choeroporpa*, Dyar, the members of which are small and difficult to separate on external characters ; the seventh appears to belong to *Mochlostyrax*, Dyar and Knab, a closely allied sub-genus.

## *Culex* (*Choeroporpa*) *innominatus*, sp.n.

MALE

*Head.* Occiput covered with loose, flat, whitish scales with golden reflections ; except a small median triangular area, widest behind, with narrow, curved, whitish scales ; flat scales continuous in front. Upright, forked scales intermixed with the flat ones above, yellow on anterior, black on posterior half. Palpi exceeding the proboscis by the length of the last segment and almost one-half the length of the penultimate segment. Proboscis and palpi clothed with blackish-brown scales. *Thorax.* Prothoracic lobes with yellowish setae above and black ones below. Mesonotum with integument varying from ochraceous-brown to dark olivaceous-brown ; covered with golden-brown, narrow, curved scales with brassy or bronzy reflections according to the direction in which viewed. Scales at sides of ante-scutellar space and on scutellum brassy. Pleurae with a narrow strip of flat white scales on sternopleura ; one lower mesepimeral bristle present. *Legs.* Clothed with very dark, blackish-brown scales with purplish reflections ; femora pale beneath. *Wings.* Distal half of vein I,

apical halves of forks of veins II and IV and apex of vein III with dense broad scales; lateral squames (Christophers 1923) in these positions with 7 to 9 striae and the width sometimes exceeding one-third of the length (from insertion to tip). *Abdomen.* Dorsum with narrow, basal, white bands, present on segments 2 to 7 in one specimen, on segments 3 to 7 in two specimens and on segments 4 and 5 in one specimen; in the fifth specimen the abdomen was lost, but it was recorded that bands were present on all but the first segment.

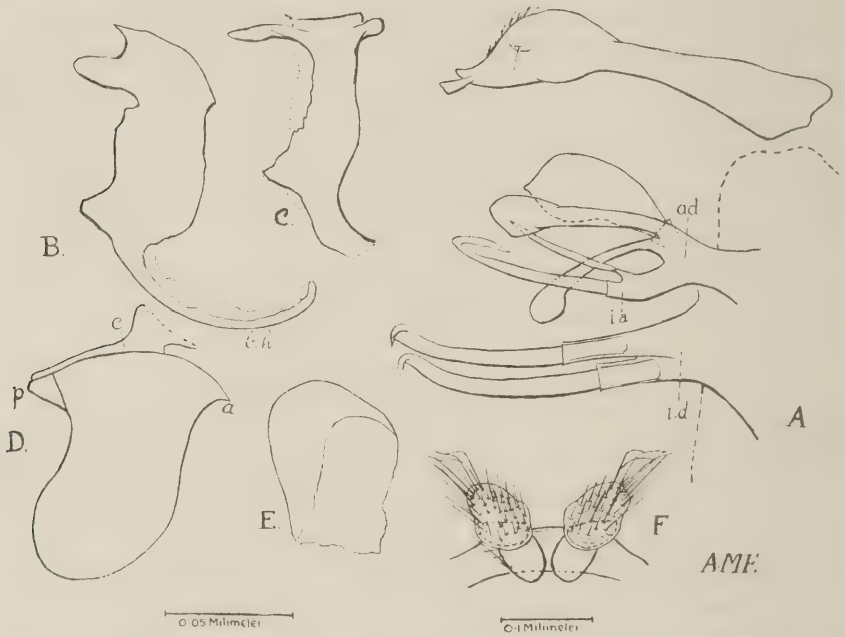


FIG. 1. *Culex* (*Choeroporpa*) *innominatus*, n.sp. Details of male hypopygium, drawn from stained preparations. A. Clasper and lobes of side-piece. *i.a.*—inner arm of outer division; *i.d.*—inner division of lobe of side-piece; *a.d.*—outer division of lobe of side-piece. B and C. Lateral plate of phallosome, lateral aspect, under different conditions of pressure. *b.b.*—basal hooks. D. Basal apodeme, flattened by pressure. *a.*—angle which articulates with paramera plate; *c.*—chitinous connection with basal arm of tenth sternite; *p.*—basal projection. E. Plate at base of side-piece. A—E to the same scale.

HYPOPYGIUM (fig. 1). The important features by which this species may be distinguished are shown in the accompanying illustration. The appendages of the outer division of the lobe of the side piece (fig. 1, A, *a.d.*) are two filaments with enlarged rounded apices and a leaf. The lateral plates of the phallosome (Christophers and Barraud, 1923) (fig. 1, B and C) even when mounted

in lateral aspect exhibit a remarkable diversity of form owing to slight differences in orientation, the shape and relative sizes of the horns or teeth appearing totally unalike in different specimens as shown by the illustrations. The basal apodeme (*ibid.*) (fig. 1, D) is subject to variation in shape according to the direction and amount of pressure put upon it. The projecting portion (*p.*) is curved at right angles to the plane of the rest of the plate, and unless the structure is completely flattened out, a greater or less degree of foreshortening results. The thin, indefinite piece of chitin (*c.*) forms a connection between the basal apodeme and a lateral projection from the base of the tenth sternite. The relative position of the 'plate at the base of the side piece' and the basal apodeme may be seen by referring to fig. 6, C.

Wing length: *c.* 2·4 mm.

Paratypes: 2 ♂♂ taken on River Amazon, 1915, Dr. A. Aiken Clark. Co-types: 2 ♂♂ taken at Palo Negro, Venezuela, 30.VIII.22, and 1 ♂ taken at Mariara, Estada Carabobo, Venezuela, 11.VIII.22, Dr. M. Núñez, Tovar.

### *Culex* (*Choeroporpa*) *clarki*, sp.n.

MALE

*Head.* Occiput entirely clothed with flat, rather closely appressed scales and a few upright, forked ones above. Flat scales appearing dark bluish-grey when viewed from above but changing to greyish white, dark sepia, or golden when the head is rotated, recalling the head scales of certain species of *Wyeomyia*. A small patch of creamy-white scales at lateral angles. Upright, forked scales consisting of a very few pale yellow ones on the anterior half, and more numerous black ones behind. Palpi exceeding the proboscis by almost the length of the last two segments. Palpi and proboscis with very dark blackish-brown scales. *Thorax.* Prothoracic lobes with long, coarse, dark brown setae. Mesonotum. Integument dark sepia, clothed with dark, bronzy, narrow, curved scales. Scutellum with integument of lobes as that of the mesonotum, but integument between the lobes tawny; scales with brassy reflections. Pleurae with two conspicuous roundish patches of flat white scales, one on each side of the upper half of the suture separating the sternopleura from the mesepimeron, and an elongated patch below on

the sternopleura. One large mesepimeral bristle present. *Legs* very dark scaled. *Wings.* Apex of vein I, apical half of the forks of vein II, apex of vein III and apices of forks of vein IV, densely clothed with short broad scales, the greatest width of the lateral squames about one-third or slightly less than one-third the length, widest on apical third and with from 5 to 7 (very rarely 8) striae. *Abdomen.* Dorsum entirely clothed with dark, blackish-brown scales. Laterally, last three segments with small basal, white, triangular spots. Venter with segments 4, 5 and 6 (and possibly 7) with proximal white bands, narrow on segment 4, nearly half the width of the segment on 5 and 6.

Wing length: *c.* 2·6 mm.

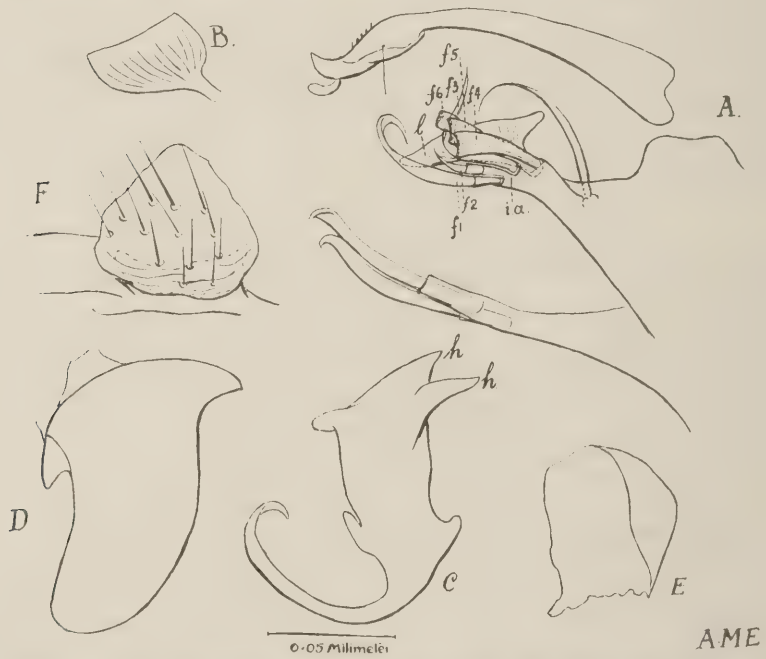


Fig. 2. *Culex* (*Choeroporpa*) *clarki*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Clasper and tip of side-piece with lobes. *f*.1 to *f*.6—filaments on outer division of lobe of side-piece; *i.a.*—inner arm of outer division of lobes; *l.*—leaf. B. Leaf of outer division in profile. C. Lateral plate of phallosome with basal hooks, lateral aspect. *h.h.*—apical dorsal horns. D. Basal apodeme flattened by pressure. E. Plate at base of side-piece. F. One of ninth tergites.

Hypopygium (fig. 2). The enlarged distal portion of the clasper is evidently comparatively narrow, but as it appeared collapsed in all the preparations, it is possible that it is really wider than as

shown in fig. 2, A. The appendages of the outer aspect of the outer division of the lobe of the side piece are somewhat complicated. Arising next to the inner arm is a rather long pointed and curved filament (*f*.3) partly hidden (in fig. 2, A) by the three curved filaments (*f*.4, *f*.5, and *f*.6), which are superimposed one above the other ; the striated leaf (*l*.) arises from a large insertion, has the surface undulating, and projects almost at right angles to the axis of the lobe and the other appendages. In consequence of this, the true shape of the leaf cannot be seen in preparations mounted to show the filaments ; fig. 2, B, was therefore drawn from the specimen when orientated to display the leaf, before mounting in the position shown in fig. 2, A. The two dorsal horns of the phallosome are large and prominent and the small projection on the ventral side near the base seems to be a constant feature. The ninth tergites are separated by a distance slightly less than the width of one of them (fig. 2, F).

Wing length : *c*. 2·6 mm.

Paratypes : 4♂♂ taken on River Amazon, 1915, Dr. A. Aiken Clark.

### *Culex* (*Choeroporpa*) *tovari*, sp.n.

MALE

*Head*. Occiput mostly covered with flat, whitish scales with pale, yellow reflections ; whitish, narrow, curved scales occupying a median triangular area, widest behind, converging to a point in front where a tuft of narrow, curved scales projects between the eyes. Palpi and proboscis with dark brown scales ; somewhat denuded proximally. Last segment of palpi wanting, penultimate segment extending beyond the proboscis by slightly less than half its own length. *Thorax*. Prothoracic lobes mostly denuded. Mesonotum shining, dark olivaceous-brown ; almost entirely denuded, but a few isolated yellowish, narrow, curved scales remaining. Scutellum tawny-brown with a few brassy, narrow, curved scales. Pleurae with a roundish patch of flat, white scales on the sternopleura above and another on the upper part of the mesepimeron, also an elongate patch before the posterior margin of the sternopleura below ; one lower mesepimeral bristle present. *Legs*. Mostly wanting ; one mid-leg complete ; dark brown scaled. *Wings*. Lateral squames on distal parts of veins II, III, and IV, markedly

narrower than in the two preceding species, the usual number of striae being only five and the length from three-and-a-half to four times the greatest width. *Abdomen.* Dorsum: segments 2 to 7 with narrow basal, whitish bands, expanding laterally. Venter with white scales at bases of segments.

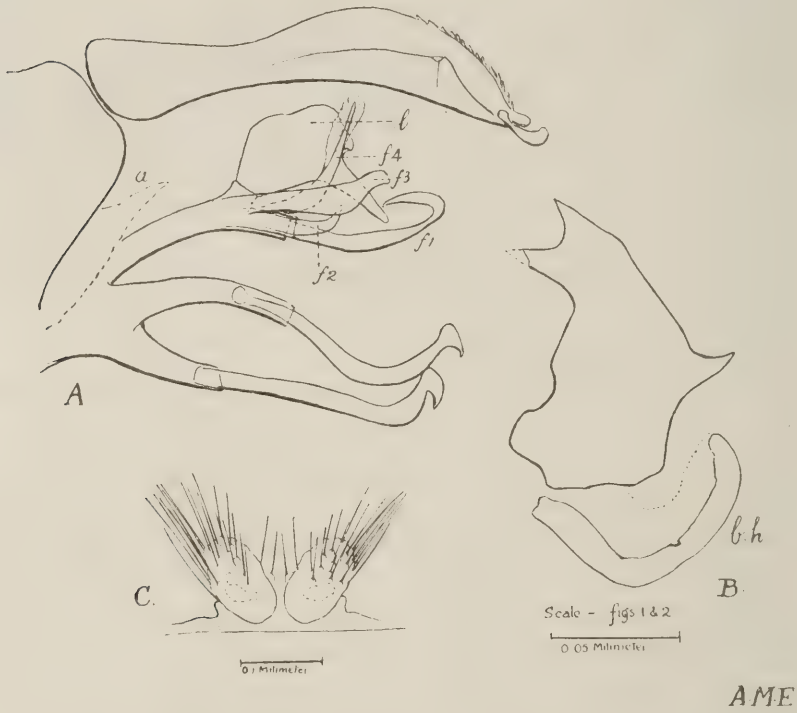


FIG. 3. *Culex* (*Choeroporpa*) *tovari*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Clasper and lobes of side-piece. *a.*—artefact; *f.*1 to *f.*4—filaments of outer division of lobe of side-piece; *l.*—leaf. B. Lateral plate of phallosome, lateral aspect with basal hooks (*b.b.*) detached. C. Ninth tergites. A and B to the same scale.

HYPOPYGIUM (fig. 3). The leaf on the outer aspect of the outer lobe of the side piece (fig. 3, A), is markedly asymmetrical and undulating; distally the margin is curled so that the outline changes considerably with slight changes in the orientation. A marked feature is the striated membranous expansion surrounding the apical part of the filament (*f.*4). The short filament (*f.*2) is closely associated, if not fused, basally with the long filament (*f.*1)

so that under a low magnification it appears to be a small basal branch of the latter. The basal apodemes are not well displayed in the slide, they appear to be rather wider distally than in the preceding species (fig. 2, D). The plate at the base of the side piece resembles that of *C. manaosensis* (fig. 5, D).

Wing length: *c.* 2·8 mm.

Type: one ♂ from Palo Negro, Venezuela, 30.VIII.22, Dr. M. Núñez Tovar.

*Culex* (*Choeroporpa*) *gordoni*, sp.n.

MALE

*Head.* Occiput clothed with flat, whitish scales with pink and brassy reflections; the numerous upright, forked scales, yellow in front, black behind. One palp present measuring 1·6 mm., the last two segments equal in length; scales brown. Proboscis incomplete. *Thorax.* Prothoracic lobes with blackish integument. Mesonotum with integument very dark blackish brown (dark olivaceous at sides with magnification of about forty times); clothed with narrow, curved, bronzy scales; bristles long, blackish. Scutellum olivaceous brown, with dull greyish-yellow scales. Pleurae very dark blackish grey; one lower mesepimeral bristle present. *Legs.* Dark brown scaled. *Wings* with the lateral squames on the apical parts of veins II, III and IV, rather broad and dense; widest type of scales in these positions with eight striae, width sometimes more than one-third of the length. *Abdomen.* Scales of dorsum brown with coppery reflections, segments 4, 5, 6 and 7 with small lateral, triangular, white spots.

HYPOPYGIUM (fig. 4). The most characteristic feature is the greatly expanded apex of the phallosome plate (fig. 4, B) which is furnished with a double row of teeth along its distal edge. The phallosome is very closely associated with the parameral plates (not shown in the figure). The outer division of the lobe of the side piece is furnished on its outer aspect with four filaments: *f.*1, *f.*2, *f.*3 and *f.*4, the last named being almost obscured (in the figure) by the others. The basal apodeme is very similar to that of *C. clarki* (fig. 2, D). Tenth sternites with about twelve very delicate teeth.

Wing length: *c.* 2·1 mm.

Type: ♂ bred from a pool at the Bosque, Manáos, 29.XII.21, Dr. R. M. Gordon.

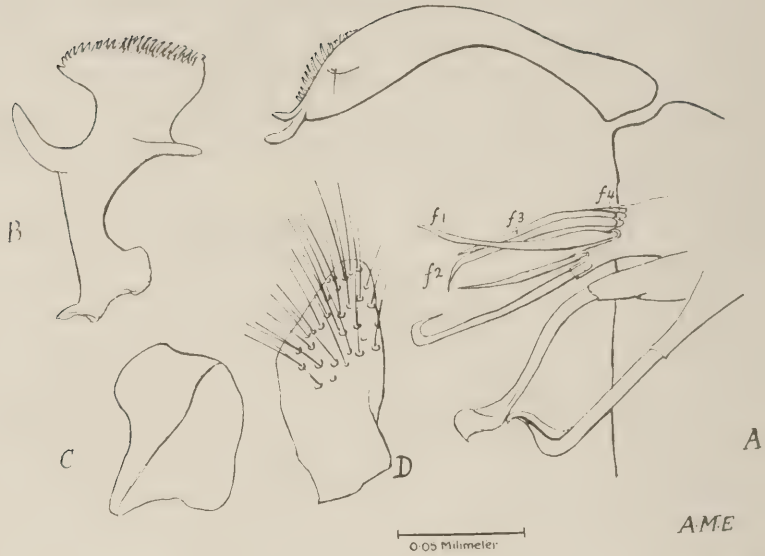


Fig. 4. *Culex* (*Choeroporpa*) *gordoni*, sp.n. Details of male hypopygium, drawn from unstained preparations. A. Apex of side-piece with clasper and lobes. *f*. 1 to *f*. 4—filaments on outer aspect. B. Lateral plate of phallosome, lateral aspect, basal hooks omitted. C. Plate at base of side-piece. D. One of the ninth tergites.

This species evidently comes near to *C.* (*Choeroporpa*) *educator*, Dyar and Knab, but I have hesitated to identify it with that species because, although the description of the mesosome (Dyar, 1920) might possibly apply to the Manáos specimen, if Dyar's figure (1918, Pl. IV, fig. 17) represents this structure in lateral view, the two species are clearly distinct.

### *Culex* (*Choeroporpa*) *manaosensis*, sp.n.

Male

*Head.* Occiput covered with flat whitish scales with metallic reflections except a triangular area of narrow, curved, whitish scales converging to a median tuft projecting between the eyes; upright, forked scales numerous, black. Palpi and proboscis covered with very dark, blackish-brown scales. Last segment of each palp missing, but penultimate segment extending beyond the proboscis by about one-half of its own length. Long hairs of antennae blackish-brown. *Thorax.* Prothoracic lobes very dark, with black bristles. Mesonotum with integument shining blackish-

brown ; scales bronzy ; that of scutellum and ante-scutellar space olivaceous-brown. Pleurae olivaceous-brown, a row of flat white scales at posterior border of sternopleura ; bristles black, rather fine, one lower mesepimeral bristle. *Legs.* Fore and mid legs with blackish scales, hind legs missing. *Wings* with widest lateral squames on veins II, III and IV mostly with seven, rarely with eight striae. *Abdomen.* Dorsum with blackish-brown scales ; all but first three segments with small, triangular, lateral, whitish spots. Venter with basal, whitish bands.

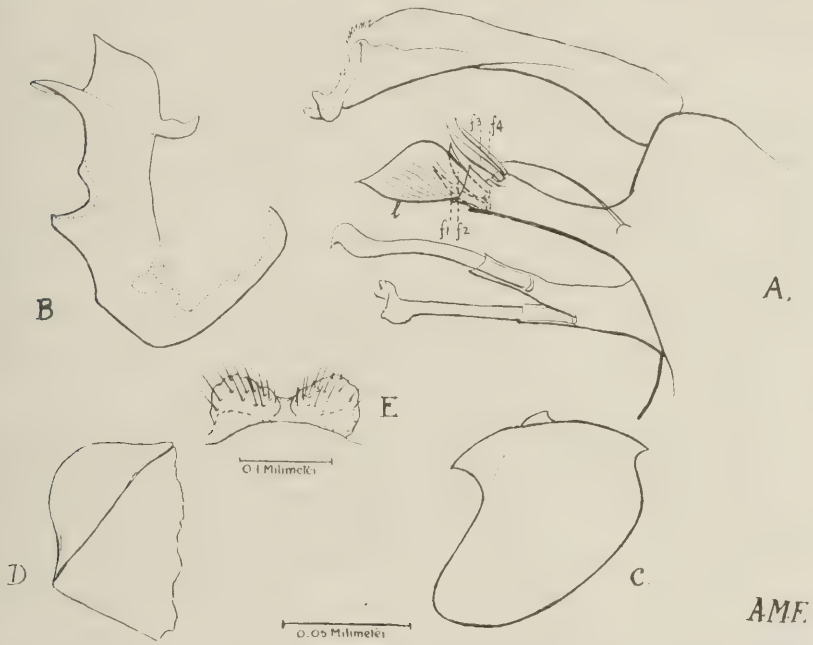


FIG. 5. *Culex* (*Choeroporpa*) *manaosensis*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Apex of side-piece with clasper and lobes ; *f.* 1 to *f.* 4—filaments of outer division ; *l.*—leaf. B. Lateral plate of phallosome with basal hooks, lateral aspect. C. Basal apodeme, flattened by pressure. D. Plate at base of side-piece. E. Ninth tergites. A—D to the same scale.

HYPOPYGIUM (fig. 5). The leaf borne by the outer division of the lobe of the side piece (fig. 5, A, *l.*) is stiff and erect with a very prominent insertion ; in the figure it obscures two of the four short, pointed filaments (*f.*1 and *f.*2) which are indicated by dotted lines. The tenth sternites have about twelve teeth.

Wing length : *c.* 2·5 mm.

Type : one ♂ taken on the Wharf, Manáos, 5.XII.23, Dr. A. Aiken Clark.

*Culex* (*Choeroporpa*) *thomasi*, sp.n.

MALE

*Head.* Occiput covered with flat, whitish scales with yellow reflections; except a median triangular area, widest behind, converging to a point in front occupied by whitish, narrow, curved scales, a tuft of narrow, curved scales projecting between the eyes. Upright, forked scales yellow in front, black behind. Palpi and proboscis covered with rather light, brown scales; palpi exceeding proboscis by the length of the last segment and half the preceding one. *Thorax.* Prothoracic lobes with blackish bristles. Mesonotum with ochraceous-brown integument and golden, narrow, curved scales. Scutellar scales brassy, bristles brown with golden reflections. Pleurae purplish-brown, light tawny around sutures, partly denuded, a row of flat, white scales along the posterior border of the sternopleura; one lower mesepimeral bristle. *Legs* unbanded, clothed with brown scales with coppery reflections. *Wings* with the widest lateral squames on veins II, III and IV with seven striae and the greatest width in many cases more than one-third of the length. *Abdomen.* Dorsum with dark brown scales and on segments 2 to 6, well defined, whitish, basal bands, narrow on second segment; segments 7 and 8 with irregular, basal, whitish bands.

HYPOPYGIUM (fig. 6). The claspers have not been figured, as the distal portions are collapsed in the preparation. In a sketch made before mounting, the distal third is rather abruptly widened, the eye-like spine and the sub-apical appendage are present, and the narrow terminal portion of the clasper is upturned as in most of the species here dealt with.

Wing length: *c.* 2·2 mm.

Type: one ♂ bearing the data 'Swamp water from Amatory, Manáos, Dr. H. Wolferstan Thomas,' 1910.

This specimen was determined on external characters as *Culex* (*Melanoconion*) *humilis*, Theo., but as the hypopygial characters showed that it was not a *Melanoconion*, the determination seemed open to doubt. Mr. F. W. Edwards kindly compared the external characters with the type of which he says 'only the head and wing are much use for comparison.' Mr. Edwards mentioned certain differences between the two specimens, and in view of these differences, and the condition of the type specimen, it seems safer

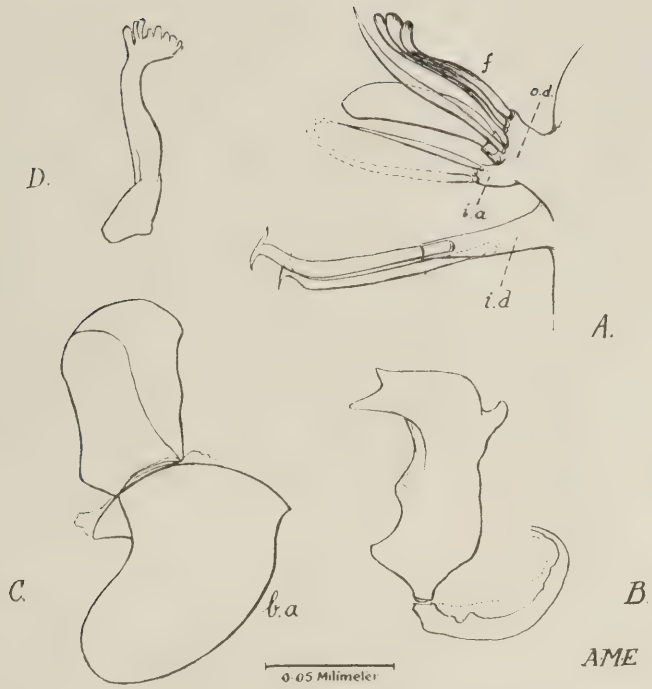


FIG. 6. *Culex* (*Choeroporpa*) *thomasi*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Lobes of side-piece. *i.a.*—inner arm of outer division ; *i.d.*—inner division ; *f.*—group of three filaments ; *o.d.*—outer division. B. Lateral plate of phallosome with basal hooks, lateral aspect. C. Basal apodeme (*b.a.*) and plate at base of side-piece. D. Tenth sternite.

to regard the specimen from Manáos as a distinct species. The fact that the type was taken at San Paulo is an additional reason for adopting this course.

Another specimen determined as *M. humilis*, Theo. was a true *Melanoconion*, which I have identified with *C.* (*Melanoconion*) *dunni*, Dyar. Mr. Edwards found that it was specifically distinct from the type of *M. humilis*, Theo.

## *Culex innovator*, sp.n.

MALE

*Head.* Occiput mostly covered with flat, whitish scales, with pale yellowish and pink, metallic reflections, appearing bluish grey in certain lights. A broad, median patch of narrow, curved, whitish scales behind extending less than half-way to the anterior margin.

Upright, forked scales all dark. Palpi and proboscis brown scaled. Palpi exceeding the proboscis by nearly the length of the last two segments. *Thorax.* Prothoracic lobes tawny with brown bristles. Mesonotum with integument bright ochraceous-brown, covered with very small, dark-brown, narrow, curved scales with golden reflections. Scutellum tawny, scales with brassy reflections. Pleurae pale fawn-coloured ; an elongate patch of flat, white scales along the posterior border of the sternopleura, one lower mesepimeral bristle. *Legs* dark-brown scaled. *Wings* with widest lateral squames not as broad as in *C. innominatus*, those on veins II, III and IV not exceeding six striae and having the greatest width less than one-third of their length. *Abdomen* dark-brown scaled above, segments 4, 5 and 6, with small, basal, lateral, triangular, pale spots, segment 7 with basal, lateral, pale spots tending to form an irregular band, segment 8 variable.

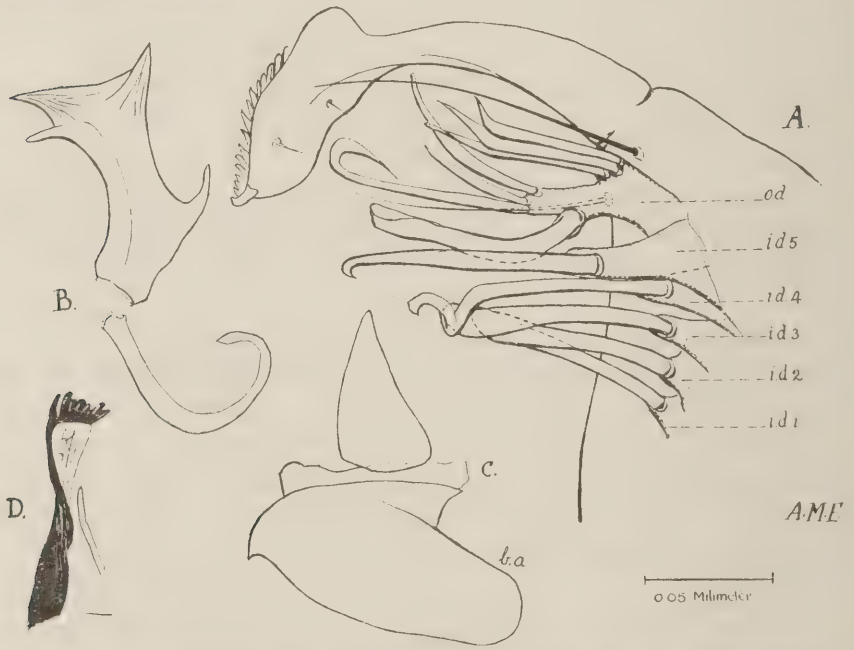


FIG. 7. *Culex innovator*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Apex of side-piece with clasper and lobes. *i.d.* 1 to *i.d.* 5—inner divisions of lobe of side-piece ; *o.d.*—outer division. B. Lateral plate of phallosome with basal hooks, lateral aspect. C. Basal apodeme and plate at base of side-piece. D. Tenth sternite.

HYPOPYGIUM (fig. 7). The most marked feature is the greatly sub-divided inner division of the lobe of the side-piece. It consists

of five distinct divisions (*i.d.* 1 to *i.d.* 5) arranged obliquely to the axis of the side-piece. The three innermost divisions (*i.d.* 1 to *i.d.* 3) bear at their extremities long, flattened filaments gradually widening distally and obliquely rounded. The extremities of these filaments lie in close proximity, so that it is difficult to display them separately in mounting them. The fourth division (*i.d.* 4) alone bears a filament or rod similar to those borne on the inner division of the side-piece in species of *Choeroporpa* (figs. 1 to 6, A). The striated appearance of the chitin forming the teeth of the plates of the phallosome (fig. 7, B) appears to be a constant feature.

Wing length: *c.* 2·4 mm.

Paratypes: three ♂♂ from the River Amazon, 1915, Dr. A. Aiken Clark.

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# NOTES ON SOME AFRICAN CERATOPOGONINAE—SPECIES OF THE GENUS *LASIOHELEA*

BY

A. INGRAM

AND

J. W. S. MACFIE

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PLATE XXII

In the collection of Ceratopogonine midges made by us in West Africa, mainly between the years 1919 and 1922, were a number of specimens (including one male) which appear to us to belong to the genus *Lasiohelea*. In examining these specimens we have had the advantage of comparing them with named examples of *L. stylifer* presented by Dr. Lutz to the Liverpool School of Tropical Medicine, and with the co-types of *L. lefanui* (Carter). The majority of our specimens are undoubtedly *L. lefanui*, but others appear to be new species and are here briefly described.

It is extremely difficult to define the genus *Lasiohelea*, or to state precisely in what respects it differs from the genera *Forcipomyia* and *Atrichopogon*. Kieffer who erected the genus in 1921 for his species *Atrichopogon pilosipennis*, gives only the following definition : hairiness of the wings as in *Forcipomyia* ; empodium as long as the claws, with short hairs ; palps having the last segment long ; otherwise as in *Atrichopogon*. We have not had an opportunity of examining *L. pilosipennis*, but we have examined *L. stylifer* which is generally admitted to belong to the same genus, and as regards this species at any rate, Kieffer's definition is scarcely adequate, for in it the hairiness of the wings is less dense than in *Forcipomyia* and of a somewhat different character, the hairs on the empodium are not

short, the last segment of the palp is scarcely, if at all, longer than the penultimate segment, and in other respects (*e.g.*, the size of the microtrichia and the form of the radial cells of the wing) there are marked differences between it and *Atrichopogon*.

More recently (1922) Edwards has published an interesting note on the genus, in which he refers to its kinship with *Atrichopogon* and *Forcipomyia* more specifically than does Kieffer. *Lasiohelea* resembles *Atrichopogon*, he says, in the structure of the antennae and in wing venation, having a very long second radial cell (which however is narrower than in *Atrichopogon*) and an obliterated first radial cell ; it resembles *Forcipomyia* in having the wings rather densely clothed with macrotrichia, but the hairs are less close-lying than in *Forcipomyia* and there are bare lines adjoining the veins, as in *Atrichopogon*. He states also that the microtrichia on the wings of *Lasiohelea* are 'smaller than those of *Atrichopogon*, but more obvious than those of *Forcipomyia*.'

We are doubtful if there is any real difference between the microtrichia of *Lasiohelea* and those of *Forcipomyia* ; in both genera they are small and slightly variable in size in different species ; but between the microtrichia of *Lasiohelea* and those of *Atrichopogon* the difference is much more marked and may serve as a ready means of separating these genera. It is more difficult to find a clear distinction between *Lasiohelea* and *Forcipomyia*. The hairiness of the wings is a variable character and the bare areas along the veins are not always present in *Lasiohelea* ; the structure of the antennae is also variable within wide limits, so that for diagnostic purposes we are apparently forced to rely on the form of the radial cells of the wing, the first cell in *Lasiohelea* being obliterated and the second long and narrow, extending beyond the middle of the wing. These cannot be considered satisfactory characteristics, for the first radial cell is not always entirely obliterated in *Lasiohelea* and *Forcipomyia* (as can readily be demonstrated by appropriate staining) and the second radial cell varies considerably in length and width in *Forcipomyia* and sometimes extends beyond the middle of the wing. The genera in which the thorax is not prolonged over the head as a hood, the empodium well developed, as long as the claws and with long hairs, and the femora and fifth tarsal segments unarmed, may perhaps, however, be distinguished as follows :—

1. Costa reaching beyond the middle of the wing, usually two-thirds its length; second radial cell usually very long; fringe on the posterior border of the wing composed of simple hairs.....2

   Costa usually not reaching beyond the middle of the wing or only slightly; second radial cell not very long; fringe on the posterior border of the wing with hairs which are usually lanceolate and sometimes pubescent or sub-plumose.....3

2. First and third veins of the wing forming two distinct cells; microtrichia large and conspicuous; fringe on the posterior border of the wing composed of a single row of alternating long and short, simple, straight hairs..... *Atrichopogon*

   First radial cell obliterated or nearly, second very narrow; microtrichia minute; fringe on the posterior border of the wing composed of long hairs between two rows of shorter, oblique, hairs..... *Lasiohelea*

3. Wings densely clothed with hairs or scales.....4

   Wings with microtrichia only. (Fifth segment of palp sub-spherical, fourth long; costa not reaching beyond the middle of the wing; first tarsal segment of hind legs half the length of the second)..... *Microhelea*

4. Scales on wings and legs..... [*Lepidohelea*]

   Without scales..... *Forcipomyia*

So far as we can determine from the examination of our specimens collected in West Africa, and of the examples of *L. stylifer* and *L. lefanui* in the collection of the Liverpool School of Tropical Medicine, the following are the chief characters of the genus *Lasiohelea*.

### *LASIOHELEA*, Keiffer, 1921

*Head.* Eyes bare or more or less hairy; broadly contiguous above, but with the facets sometimes rather widely separated. Palpi sometimes with the fifth segment longer than the fourth; third segment with or without a sensory pit. Mouth parts well chitinised. Labium fleshy, somewhat similar to that of *Culicoides*. In *L. lefanui* the labrum tapers distally, ends in a sharp point, and is not armed with teeth; the mandibles are strongly chitinised, taper in the distal third, end in sharp points, and bear a row of

about twenty-five small teeth along the distal margin; the maxillae are shorter than the mandibles and less highly chitinised, and bear fewer teeth, about fifteen, distally; and the hypopharynx is strongly chitinised, tapers distally, and ends sharply. *Antennae* of female with flagellum segments sometimes forming an almost continuous series, sometimes with the basal segments short and broad and the last five segments much elongated, an abrupt change of shape taking place between the tenth and eleventh segments. In none of the species examined by us were the basal segments so short and broad ('transversaux') as they often are in *Atrichopogon*. Terminal segments of antenna not definitely sculptured, but in some species irregularly chitinised and having in consequence a patterned appearance. Whorls of hairs on the basal segments composed of about eight to twelve hairs; large spines usually only a little stouter than the hairs, about as long as the segments, straight or nearly, and tapering to more or less pointed extremities. *Thorax*. Scutellum bearing few bristles and hairs, the former in a transverse row, often with the most lateral bristle on each side somewhat apart from the rest. *Wings* usually short and broad, with a rounded distal extremity. Fringe on the posterior part of the wing composed of long hairs between two rows of shorter oblique hairs, not as in *Atrichopogon* of practically a single row of alternating long and short straight hairs. Fringe hairs apparently simple and straight. Border of wing thickened, especially anteriorly; the gap just beyond the end of the costa distinct. Microtrichia small, as in *Forcipomyia*, smaller than in *Atrichopogon*. Macrotrichia covering the wings more or less completely and densely, but not so densely as in *Forcipomyia*, slightly curved, slender, hair-like, tapering to very sharply pointed ends. Those on the costa and the anterior veins sometimes broader, scale-like. There may or may not be definite bare areas along the veins, as in *Atrichopogon*. Costa reaching well beyond the middle of the wing, usually two-thirds the length. Sub-costa strongly developed, bearing stout hairs or scale-like hairs. First radial cell usually obliterated and at most very narrow and slit-like. Second radial cell very long and usually very narrow. Cross-vein oblique. Petiole of the fourth vein short, almost obsolete, rami obsolete proximally and sometimes very indistinct for their entire lengths. Bifurcation of the fifth vein

proximal to the level of the end of the costa, the rami terminating at the wing margin, the one proximal and the other distal to this level. *Legs.* First tarsal segment longer than the second on all the legs ; on the hind legs usually two and a half to three times as long, but in one species examined by us, shorter, a little less than twice the length of the second. Terminal tarsal segments sub-cylindrical. In some species the tarsal segments bear fringed, striated scales in addition to hairs. Claws short, equal, usually rather stout and having a thickening about the middle. Empodium nearly as long as the claws, hairy, the hairs actually long, but curved and thus in some views appearing short. *Abdomen.* Well clothed with hairs and often with a small admedian tuft on each side of the eighth sternite. There may be either a single spermatheca or two spermathecae. The hypopygium of the single male examined by us resembled in some respects that of *Forcipomyia ingrami.*

## Key to the West African Species of the genus *Lasiohelea*.

1. With scales on the legs.....2
   Without scales.....5
2. Hind legs with first tarsal segment less than twice the length of the second..... *L. brevitarsata*, sp.n.
   Hind legs with first tarsal segment two to three times the length of the second.....3
3. Third segment of palp without a sensory pit..... *L. nigeriae*, sp.n.
   Third segment of palp with a sensory pit.....4
4. Spermatheca single, sub-spherical, the basal portion not chitinised..... *L. lefanui* (Carter) var. *squamipes*,* var. n.
   Spermathecae two, pyriform..... *L. caliginosa*, sp.n.
5. Eyes hairy..... *L. litoraurea*, sp.n.
   Eyes bare.....6
6. Antenna with the segments of the flagellum forming an almost continuous series, with no abrupt change of shape between the tenth and eleventh segments ..... *L. inconspicuosa*, sp.n.
   Antenna with the last five segments much elongated, with an abrupt change of shape between the tenth and eleventh segments..... *L. lefanui* (Cart.)

* This East African species is included here because it is very closely allied to *L. lefanui* and may eventually be found to occur also in West Africa.

*LASIOHELEA NIGERIAE*, sp.n.

Length of body* (two females and one male), 1·3 mm. to 1·4 mm.; length of wing, 0·9 mm. to 1·1 mm.; greatest breadth of wing, 0·3 mm. to 0·35 mm. The male is slightly longer than the females, and its wings slightly longer and narrower.

A dark brown insect with yellowish legs, closely resembling *L. lefanui* (Carter), but differing from the three co-types of this species in the following respects. *Head.* Eyes bare above, slightly hairy

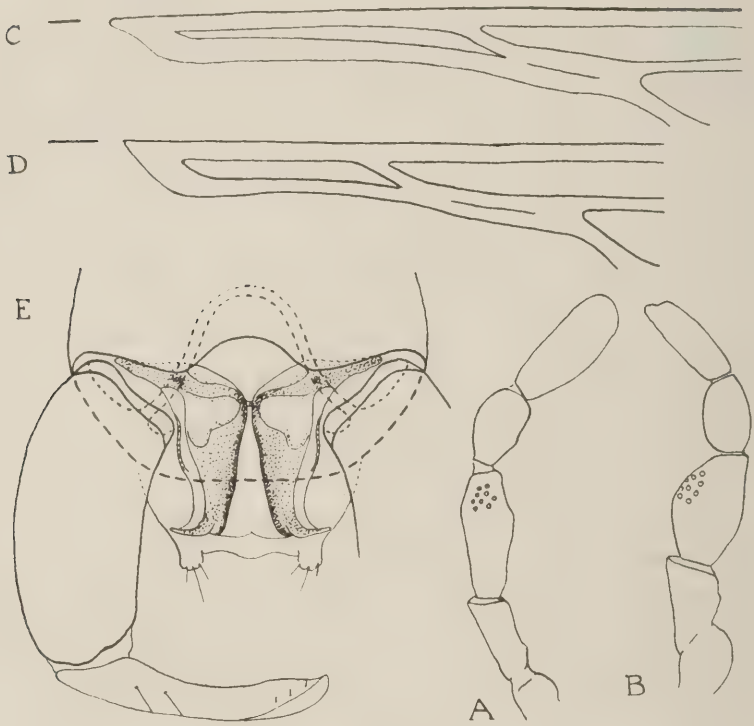



FIG. 1. *Lasiohelea nigeriae*, sp.n.: A. palp, ♂; B. palp, ♀; C. radial cells, ♀; D. radial cells, ♂; E. hypopygium, ♂, ventral view. [C. and D. × c. 265, others × c. 400.]

below and at the inner margins; broadly contiguous above in both sexes. Palpi (fig. 1, A and B) in both sexes with the fifth segment longer than the fourth; in the female the third segment a little inflated in the middle, in the male hardly at all, in both sexes without a sensory

* In all cases this measurement is taken from the anterior margin of the thorax to the tip of the abdomen of specimens mounted in pure carbolic acid.

pit but bearing sensory hairs on the inner anterior aspect. *Antennae.* In the female segments 4 to 10 similar to those of *L. lefanui*, the length and breadth of segments 4 and 10 in one specimen measuring 6 by 8, and 9 by 6 units* respectively ; segments 11 to 15 elongated, rather longer than in *F. lefanui*, the lengths of the eleventh and fifteenth segments in one specimen measuring 20 and 25 units respectively. The combined length of segments 11 to 15 greater than that of segments 3 to 10 and twice as great as that of segments 4 to 10, the three actual measurements in one specimen being 109, 61, and 51 units respectively. In the single male in our possession the antennae are damaged, the terminal segments missing. Torus very large and very dark brown ; flagellum segments brown. Segments 4 to 11 all about the same length, 12 units, but rapidly diminishing in greatest breadth from a little over 10 units in the case of the fourth segment to a little over 5 units in that of the eleventh. *Thorax.* Scutellum with fewer small hairs, about six in the female and one or two in the male. *Wings* (Pl. XXII, fig. 1) similar to those of *L. lefanui* but with the hairs on the costa and the anterior veins dark brown, lanceolate, scale-like. Bare areas along the veins well marked. Wings not so densely clothed with macrotrichia as in *L. lefanui*. At the base of the wing, between the fourth and fifth veins, one or two rows of macrotrichia ; at the level of the bifurcation of the fifth vein, two rows between the fourth and fifth veins, three rows between the rami of the fourth vein, and two rows between the third and fourth veins. Microtrichia very minute. The tip of the wing rounded but not as broad as the broadest part of the wing. In the male the wings are slightly longer and narrower than in the female, costa reaching nearly to two-thirds of the length of the wing from the base (35 : 58), first radial cell obsolete, second long and narrow but neither so long nor so narrow as in the female (fig. 1, C and D), bifurcation of the fifth vein a little proximal to the end of the costa. *Legs.* Tarsal segments somewhat infuscated, bearing numerous striated scales which are most conspicuous on the distal segments. First tarsal segment of all the legs, over twice the length of the second, the actual measurements of the first two tarsal segments of the fore, middle, and hind legs of one female being 50 and 21, 47 and 22, and 59 and 26 units respectively.

* The unit referred to is $3{\cdot}7\mu$.

*Abdomen* bearing lanceolate, scale-like hairs similar to those on the wing, in addition to ordinary hairs. Abdomen of the male rather dark brown, narrow, with a conspicuous, dark brown hypopygium. Spermatheca single, sub-spherical, of the same form as that of *L. lefanui*, diameter about 60$\mu$, the base not chitinised ; the diameter at the level where the chitinisation ceases about 25$\mu$.

HYPOPYGIUM (fig. 1, E). *Ninth segment* dark brown : tergite hairy, with a posterior extension similar to that of *Forcipomyia* ; sternite with fairly numerous hairs restricted to the anterior part, slightly notched in the middle line posteriorly. *Forceps* well developed : side-pieces about twice as long as broad, moderately hairy, their proximal ends with a highly chitinised rim ; claspers brown, well chitinised, their ends somewhat spoon-shaped. *Harpes* : there are no posteriorly projecting plates. The strongly chitinised dorsal root-like processes arising at the bases of the side-pieces are long and join anteriorly across the middle line forming a wide arch somewhat similar to that of *Forcipomyia ingrami*. *Aedoeagus* a highly complicated structure, partly chitinised, partly membranous ; in ventral view the chitinised portions appear as two roughly triangular objects with their apices apposed in the middle line a little posterior to the margin of the ninth sternite. In lateral view there is seen to be on each side at the distal end a process directed dorsally.

NIGERIA : Calabar, February, 1922, 1 ♂, 2 ♀♀ (Dr. E. C. Braithwaite).

Four specimens (♀♀) of *L. stylifer*, named by Dr. Lutz, in the collection of the Liverpool School of Tropical Medicine show scales similar to those of the species described above ; but on the three co-types (♀♀) of *L. lefanui* in the same collection, and on some specimens collected by us in Nigeria and the Gold Coast, and on others sent to us by Dr. J. R. C. Stephens from Ilorin, Nigeria, which appear to us to be *L. lefanui*, we are unable to find any such scales. The species described above, therefore, resembles *L. stylifer* even more closely than *L. lefanui*, but there are certain differences, *e.g.*, in the palps which in *L. stylifer* have a large sensory pit in the third segment and the fourth and fifth segments subequal, and in the absence of a comparison of the genitalia of the male, we think the two species must be regarded as distinct although undoubtedly closely allied.

### *LASIOHELEA BREVITARSATA*, sp.n.

Length of body (one female), 0·9 mm. ; length of wing, 0·8 mm. ; greatest breadth of wing, 0·3 mm. Resembling the preceding species, *L. nigeriae*, in almost every respect, but smaller and with relatively shorter first tarsal segments.

*Head.* Eyes very slightly hairy, as in *L. nigeriae.* Palpi as in *L. nigeriae. Antennae* similar to those of *L. nigeriae* but smaller. Segments 4 and 10 measuring approximately 6 by 6, and 7·5 by 5 units respectively. Segments 11 to 14 sub-equal, about 15 by 5 units ; the fifteenth segment a little longer, about 20 by 5 units, and ending in a blunt stylet. The combined length of segments 11 to 15 (78 units) greater than that of segments 3 to 10 (50 units), and not quite twice that of segments 4 to 10 (43 units). *Thorax.* Scutellum bearing a transverse row of eight bristles and a few (six) smaller hairs as in *L. nigeriae. Wings* (Pl. XXII, fig. 2). Shape of wing and distribution of macrotrichia as in *L. nigeriae.* Costa reaching nearly two-thirds of the wing length (25 : 43). Halteres somewhat infuscated. *Legs* bearing scales as in *L. nigeriae.* First tarsal segment of the fore and middle legs about twice, and of the hind legs a little less than twice the length of the second ; the actual measurements of the first and second tarsal segments of the fore, middle, and hind legs being 37 and 18, 35 and 17, and 43 and 24 units respectively. *Abdomen* as in *L. nigeriae.* Spermatheca single, sub-spherical, of the same form as in *L. lefanui*, diameter about 50$\mu$, the extreme base not chitinised, the diameter at the level where the chitinisation ends, about 15$\mu$.

GOLD COAST : Accra, 1920, 2 ♀♀, taken in the laboratory.

### *LASIOHELEA LEFANUI*, (Carter) var. *SQUAMIPES*, var. n.

In a former paper (1923) we stated that we had been unable to detect any differences by which *L. stylifer* could be distinguished from the African species which we described as *L. lefanui.* In connection with the present work we have had occasion to re-examine the co-types of *L. lefanui* and specimens of *L. stylifer* named by Dr. Lutz. Whilst agreeing in most respects these specimens differ slightly in a number of minor points which may be of less than specific importance, and more notably in that whereas *L. stylifer*

has numerous scales on the legs, the co-types of *L. lefanui* appear to be entirely without scales.

In a small collection of African Ceratopogoninae kindly lent to us for examination by Dr. G. A. K. Marshall of the Imperial Bureau of Entomology, were a number of specimens of *Lasiohelea*. In one tube, to which unfortunately no label indicating locality was attached, but which probably came from Zanzibar (Dr. W. Mansfield Aders), were several specimens of a species to which brief reference must be made. These insects resemble *L. lefanui* in almost every respect, but on the legs there are a few narrow scales (not so many, however, as in the specimens of *L. stylifer* we have examined). On the wings the bare areas along the veins are distinct. At the base of the wing, between the fourth and fifth veins, are two or three rows of macrotrichia ; and at the level of the bifurcation of the fifth vein, about four rows between the fourth and fifth veins, three or four between the rami of the fourth vein, and two or three between the third and fourth veins. The distribution of macrotrichia is thus much as in *L. lefanui*, but more sparse than in *L. stylifer*, in which, in the examples examined by us, there are six or seven rows at the base between the fourth and fifth veins, and at the level of the bifurcation of the fifth vein about five rows between the fourth and fifth veins, four or five between the rami of the fourth vein, and three or four between the third and fourth veins. They differ from the species we have described above as *L. nigeriae* in having the wings more hairy, and the palps with the third segment furnished with a large but shallow sensory pit, and the last two segments sub-equal.

In the present state of knowledge of the genus, and in view of the apparent great variability of *L. lefanui*, we think it best to regard these specimens as a variety of that species and propose for them the name *Lasiohelea lefanui* (Carter) var. *squamipes*.

### *LASIOHELEA CALIGINOSA*, sp.n.

Length of body (one female), 1·2 mm. ; length of wing, 1·0 mm. ; greatest breadth of wing, 0·4 mm. A very dark brown midge with dark brown legs on the tarsal segments of which are striated scales ; closely resembling *L. nigeriae*.

*Head* very dark brown. Eyes bare ; broadly contiguous above, the facets narrowly separated. Clypeus, proboscis, and palpi dark brown. Mandibles apparently not armed with teeth. Palpi (fig.2, A) with fifth segment only slightly longer than the fourth, third slightly inflated in the middle and furnished with a small sensory pit. *Antennae.* Torus very dark brown, sub-spherical, not hollowed out anteriorly, bearing several longish hairs. Flagellum segments dark brown, but not so dark as the torus. Basal segments of the flagellum bearing large spines which are a little stouter than the hairs, slightly curved, about as long as the segments, tapering to not very sharp points ; and whorls of about ten to twelve hairs. Third segment larger than the fourth, with a quite short stalk. Segments 4 to 10 short and broad, from a little broader than long to a little

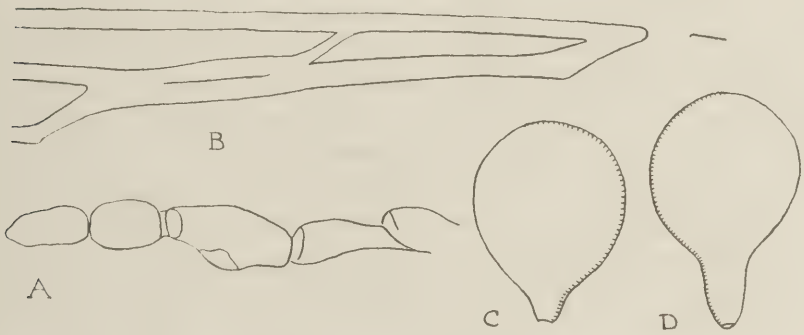



FIG. 2. *Lasiobelea caliginosa*, sp.n., ♀ : A. palp ; B. radial cells ; C. and D. spermathecae. [B. × c. 265, others × c. 400.]

longer than broad, the fourth and the tenth segments actually measuring in length and breadth 7 by 8, and 8 by 7 units respectively. Segments 11 to 15 elongated, three to nearly four times as long as broad, their lengths in one female 17, 20, 21, 22, and 27 units respectively, the terminal segment ending in a blunt sessile stylet. The combined length of segments 11 to 15 greater than that of segments 3 to 10 and about twice as great as that of segments 4 to 10, namely 107, 59, and 50 units respectively. *Thorax.* Dorsum uniformly very dark brown ; pleura dark brown. Scutellum dark brown, but not so dark as the dorsum ; bearing a transverse row of about eight bristles, and about fifteen smaller hairs. Post-scutellum

very dark brown. *Wings* (Pl. XXII, fig. 3) unadorned, but the anterior border appears darker than the rest of the wing on account of the density of the hairs in this region. Microtrichia minute. Macrotrichia covering almost the entire wing, moderately densely. Bare areas along the veins indistinct. At the base of the wing between the fourth and fifth veins three or four rows of macrotrichia ; at the level of the bifurcation of the fifth vein, about five rows between the fourth and fifth veins, about five rows between the rami of the fourth vein, and three or four rows between the third and fourth veins. The tip of the wing rounded but not the broadest part of the wing. Macrotrichia on the costa and anterior veins as in *L. nigeriae*, but narrower. Costa reaching beyond the middle of the wing (35 : 55). First radial cell obliterated, second long and narrow but not so long as in *L. nigeriae* (fig. 2, B). Bifurcation of the fifth vein well proximal to the end of the costa. Halteres with brown knobs. *Legs* rather dark brown, the tarsal segments paler brown than the proximal segments ; well clothed with shortish hairs, and bearing striated scales on the tarsi. First tarsal segment over two-and-a-half times as long as the second on all the legs, *i.e.*, measuring in one specimen 53 : 19, 51 : 18, and 63 : 22 on the fore, middle, and hind legs respectively. Claws stout, equal, about half the length of the fifth tarsal segment. Empodium long and hairy. *Abdomen* very dark brown, the venter somewhat paler brown proximally ; densely clothed with very dark hairs. Spermathecae two, very dark and highly chitinised, pyriform but somewhat dissimilar in both the females examined (see fig. 2), the total length and greatest breadth of the one, 63$\mu$ by 50$\mu$, and of the other, 77$\mu$ by 52$\mu$ in one specimen.

Gold Coast : Accra, 1920 and 1921, 2 ♀♀, taken in the laboratory.

This insect resembles closely both *L. stylifer* and the preceding species, *L. nigeriae*, but differs from both in a number of characters. For example, the third segment of the palp is furnished with a sensory pit, but it is only a small one ; the first tarsal segments of the hind legs are relatively longer, nearly three times as long as the second ; and the spermathecae are two and pyriform.

### *LASIOHELEA LITORAUREA*, sp.n.

Length of body (two females), 0·8 mm.; length of wing, 0·7 mm.; greatest breadth of wing, 0·27 mm. A small brown midge, not very dark coloured, with paler brown legs: without scales.

*Head* darkish brown. Eyes hairy; narrowing above, the margins contiguous, but the facets separated rather widely, by about 18$\mu$. Clypeus, proboscis, and palpi brown. Palpi with first segment small, second and fourth sub-equal, not quite twice as long as broad, fifth small, shorter than the fourth with which it is united rather broadly, third about as long as the fourth and fifth together, slightly inflated in the basal half, without a sensory pit, but with a shallow depression bearing sensory hairs on its inner aspect. *Antennae* (fig. 3, A) brown; torus darker than the flagellum segments. Third

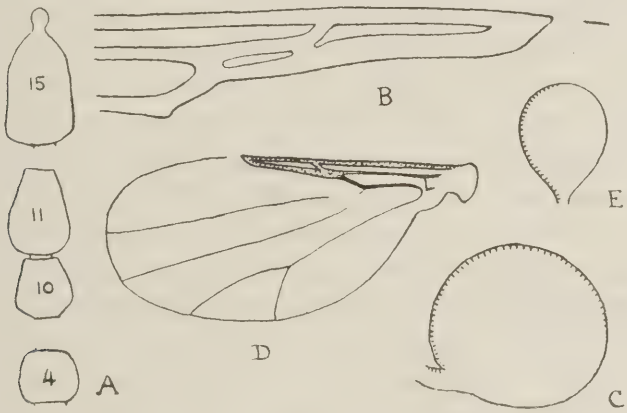



FIG. 3. *Lasiohelea litoraurea*, sp.n., ♀: A. segments 4, 10, 11, and 15, of antenna; B. radial cells; C. Spermatheca. *Lasiohelea inconspicuosa*, sp.n. ♀; D. diagram of wing venation; E. spermatheca. [D. × c. 80, B. × c. 265, others × c. 400.]

segment with a short stalk. Segments 4 to 10 sub-equal, sub-spherical, the fourth being slightly broader than long, the tenth slightly longer than broad; bearing stout spines which are about as long as the segments and are not very sharply pointed at their ends, and whorls of about twelve hairs. Segments 11 to 15 more elongated, the change of shape between the tenth and eleventh being definite; segments 11 to 14 sub-equal, length about once-and-a-half the breadth, the fifteenth slightly longer and broader and ending in a small knob. The terminal segments (11 to 15) not definitely

sculptured, but chitinised irregularly and so presenting a somewhat patterned appearance. The combined length of segments 11 to 15 about equal to that of segments 3 to 10, *e.g.*, in one specimen, 41 to 43 units. *Thorax* brown, the dorsum much darker than the pleura; sparsely clothed with hairs. Scutellum dark brown; bearing two lateral and five centro-marginal bristles, and about six small hairs. Post-scutellum dark brown. *Wings* (Pl. XXII, fig. 4) unadorned; well-clothed all over with minute microtrichia and longish hairs. Macrotrichia of the usual type, extending over practically the whole wing with the exception of the radial areas. There are, however, indistinct traces (especially along the fifth vein) of bare areas along the veins. At the base of the wing, between the fourth and fifth veins, are three or four rows of macrotrichia; at the level of the bifurcation of the fifth vein, about five rows between the fourth and fifth veins, about five rows between the rami of the fourth vein, and three rows between the third and fourth veins. It is difficult to determine exactly the number of rows because they are irregular, and because the rami of the fourth vein are very indistinct, indeed almost obsolete. The macrotrichia on the costa and anterior veins are longer and stronger than those elsewhere on the wing, but are not scale-like. The tip of the wing is rounded and very broad. Fringe well developed, composed of three rows of straight, simple hairs; the border of the wing thickened excepting for a short distance just beyond the end of the costa. Costa reaching beyond the middle of the wing, almost two-thirds of its length (22 : 36). First radial cell indistinct but not entirely obliterated, second long and narrow (fig. 3, B). Bifurcation of the fifth vein a little proximal to the end of the costa. Halteres pale brown. *Legs* brown, not so dark as the rest of the body, almost uniformly coloured; well clothed with hairs, but without scales. Femora not swollen, unarmed. Fore tibiae with a small patch of large, spine-like hairs near the apex; hind tibiae with the usual two transverse rows of bristles distally. First tarsal segment more than twice the length of the second on all the legs, on the hind legs nearly three times as long (37 : 13); distal segments short, sub-cylindrical. Claws rather slender, about half the length of the last tarsal segment. Empodium long and hairy. *Abdomen* brown, sides paler; clothed with shortish brown hairs. Spermatheca (fig. 3, C) single, sub-spherical, diameter about 50$\mu$; duct arising obliquely and chitinised for a short distance.

GOLD COAST: Accra, 27.III.1920, 2 ♀♀, taken in the laboratory; Christiansborg, near Accra, 14.II.1916, 1 ♀ (Dr. J. W. S. Macfie), 'taken in the Castle.'

### *LASIOHELEA INCONSPICUOSA*, sp.n.

Length of body (one female), 0·9 mm.; length of wing, 0·6 mm.; greatest breadth of wing, 0·27 mm. A small brown midge very similar to the preceding species (*L. litoraurea*), but differing from it in the following respects:—

*Head.* Eyes bare; facets separated only narrowly. *Antennae.* The flagellum segments form an almost continuous series, the change of shape between the tenth and eleventh segments being slight; the basal segments slightly broader than long, the distal ones slightly longer than broad, the measurements of length and greatest breadth of the fourth, tenth, eleventh, and fourteenth segments being 5 by 7, 6 by 6, 7 by 6, and 7 by 6 units respectively. The last segment is slightly longer and broader than the others, about 12 by 7 units, and ends in a blunt knob. The combined length of segments 11 to 15 slightly greater than that of segments 4 to 10, but slightly less than that of segments 3 to 10, namely, 41 units as compared with 37 and 44 units. *Thorax.* Scutellum bearing two lateral and seven centro-marginal bristles, and about twelve small hairs. *Wings* (fig. 3, D). Shape of wing and distribution of macrotrichia as in *L. litoraurea*, but the microtrichia rather larger, and the first radial cell obliterated. *Legs* as in *L. litoraurea*. *Abdomen.* Spermatheca (Pl. XXII, fig. 5 and text-fig. 3, E) single, pyriform, length about 38$\mu$, greatest breadth about 30$\mu$; the duct chitinised for a very short distance, about 3$\mu$.

GOLD COAST: Accra, 1920, 1 ♀, taken on a window in the laboratory in the evening.

### *LASIOHELEA LEFANUI*, (Carter)

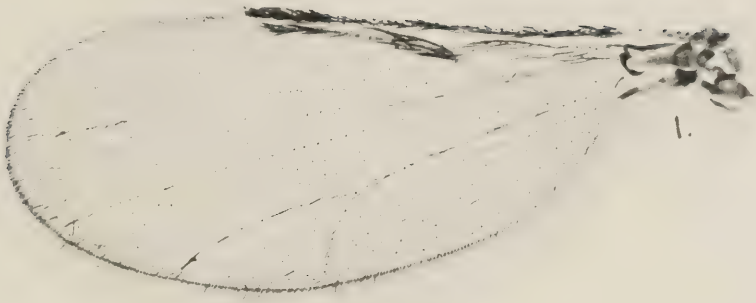
NIGERIA: Minna, 14.VIII.1910, 2 ♀♀ (Dr. J. W. S. Macfie); Ilorin, old Residency, 29.VIII.1920, numerous ♀♀ (Dr. J. R. C. Stephens), 'biting at 1 p.m.'; Mokwa, 16.XI.1921, several ♀♀ (Dr. J. R. C. Stephens), 'biting at noon.'

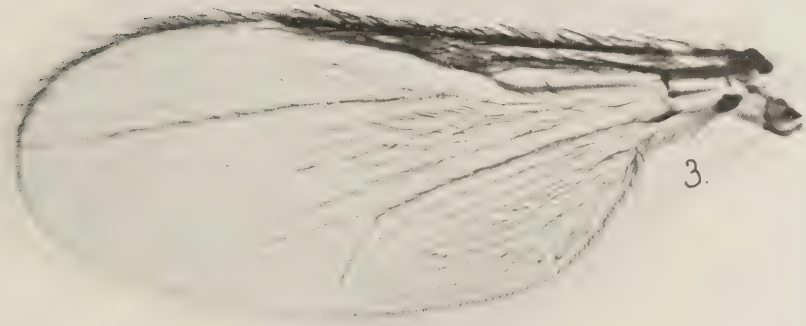
GOLD COAST: Accra, 1920, 1 ♀; Northern Territories, Bole, 26.VII.1918, Dogankade, 14.V.1918, Kulmasa, 22.VII.1918, Malowe, 29.VII.1918, Tanina, 21.VII.1918, and Ulu, 7.VII.1918, numerous ♀♀ (Dr. A. Ingram) 'taken in the act of biting.'

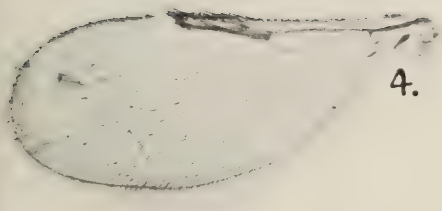
## EXPLANATION OF PLATE XXII

Fig. 1. Wing of female of *Lasiohelea nigeriae*, sp.n.
Fig. 2. ,, ,, *L. brevitarsata*, sp.n.
Fig. 3. ,, ,, *L. caliginosa*, sp.n.
Fig. 4. ,, ,, *L. litoraurea*, sp.n.
Fig. 5. ,, ,, *L. inconspicuosa*, sp.n.

Figs. 1 to 3 from unstained, figs. 4 and 5 from lightly stained preparations.





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# A REVIEW OF THE OESOPHAGOSTOMES IN THE COLLECTION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

H. THORNTON

(*From the Parasitological Department of the Liverpool School of Tropical Medicine*)

(*Received for publication September* 15, 1924)

The material available for examination was as follows :—

1. *Oesophagostomum columbianum* from ileum of a Merino sheep.
2. *Oesophagostomum columbianum* from fourth stomach and small intestine of a sheep, Nairobi.
3. *Oesophagostomum columbianum* nodules from the small intestine of a sheep, Nairobi.
4. *Oesophagostomum columbianum* from duodenum of goat.
5. *Oesophagostomum columbianum* from a water buck, Ngoa, North-eastern Rhodesia.
6. *Oesophagostomum venulosum* from goat.
7. *Oesophagostomum venulosum* from lamb, Pwllheli, North Wales.
8. *Oesophagostomum radiatum* from caecum and large intestine of zebu.
9. *Oesophagostomum dentatum* from pig, Suffolk.
10. *Oesophagostomum mwanzae* from caecum and colon of wart hog (*Phacocaerus aethiopicus*), Rhodesia.
11. *Oesophagostomum eurycephalum* from caecum and colon of wart hog, Rhodesia.
12. *Oesophagostomum simpsoni* from caecum and colon of wart hog, Rhodesia.
13. *Oesophagostomum oldi* from caecum and colon of wart hog, Rhodesia.
14. *Oesophagostomum yorkei*, n.sp. from caecum and colon of wart hog, Rhodesia.
15. *Oesophagostomum ventri* n.sp. from stomach of Brazilian wild cat.
16. *Oesophagostomum brumpti* from chimpanzee (*Anthropopithecus* sp.)

Genus *Oesophagostomum*, Molin, 1861.

It is not proposed to use the subgenera *Hysteracrum*, *Oesophagostomum* and *Proteracrum* created by Railliet (1919) for the oesophagostomes of *Artiodactyla*. The discovery of several new species of the genus *Oesophagostomum* from the roan antelope (*Hippotragus equinus*) and the wart hog (*Phacocaerus aethiopicus*) has led one to realise the limitations of Railliet's subgenera. Neither Daubney's new species *O. mwanzae*, though it agrees most nearly with the subgenus *Proteracrum*, nor *O. eurycephalum* and *O. oldi* of Goodey (1924), can be included in any of these. It seems, then, that in regard to the genus *Oesophagostomum* one is fast approaching the stage where a subgenus will hold but a single species, obviously an untenable position. Goodey says of this :

> 'We have in the genus *Oesophagostomum* a neat and compact array of species which resemble the type species of the genus (*O. dentatum*) on broad lines, as for example in possessing a mouth collar with circumoral papillae, mouth with, or in one case, without external leaf crown, a cephalic vesicle generally inflated. The spicules and gubernaculum similar in structure and appearance, genital cone built on same general plan throughout all the species and ovijector apparatus also similar in all the species.'

On these grounds it is suggested that the genus be maintained intact. Further classification, if the number of species rendered the genus unwieldy, should include, in addition to the oesophagostomes of the *Artiodactyla*, those of Primates, Man, Rodentia, Carnivora and all other oesophagostomes without regard to the animal host. Grouping according to the animal host, as Railliet has done, is unsatisfactory, for there is a close relationship between *O. ventri* from the Brazilian wild cat and *O. stephanostomun* from man, and between *O. xeri* of the ground squirrel and *O. brumpti* of man.

*Oesophagostomun columbianum* (Curtice, 1890) Stossich, 1899.

Ransom (1911) says that this worm is found in the large intestine ; but it will be noticed that of the five lots of material collected, two from Nairobi, from Natal, from Queensland and North-eastern Rhodesia, all the worms were collected from the abomasum (fourth stomach) or small intestine. It appears to be the commonest oesophagostome of ruminants in Africa and America and is responsible for the so-called ' pimply gut ' of sheep. Theiler (1921) says ' very few sheep

pass our post-mortem table in which they are absent.' He further distinguishes two fatal sequelae to a severe infection, (1) a septic infection of the serous cavities due to the rupture of one or more intestinal nodules and, (2) an invagination of the ileum. To my knowledge severe infestations due to oesophagostomes, or such sequelae, are unheard of in England.

Descriptions of this nematode are given by Ransom, and more recently, by Goodey (1924). Curtice (1890) says of the two leaf crowns, 'There are twenty-four elements in each.' Giles agrees with him, but figures the inner row as typical bidentate elements. In the specimens I have examined there seems little doubt that the internal crown is composed of 48, twice as many elements as the external. Giles probably mistook two adjoining teeth for a larger bidentate one. *O. columbianum* has not previously been recorded from the water buck.

*Oesophagostomum venulosum* (Rudolphi, 1809) Railliet, 1896.

This is the commonest oesophagostome of the domesticated animals in England. Morgan says in his survey of the Aberystwyth area of Wales, '*O. venulosum* has been frequently found in the large intestine of sheep, but only in small numbers.' Ransom gives the number of elements in the external and internal leaf crowns as not more than sixteen processes in each worm. There is no doubt that, as in *O. columbianum*, the internal leaf crown is composed of twice as many elements as the external.

*Oesophagostomum radiatum* (Rudolphi, 1803) Railliet, 1898.

Some fifteen specimens were taken from the caecum and colon of the zebu. It has not been previously recorded from this host. Other recorded hosts are *Bos taurus* and the water buffalo. In the male bursa the common stem of the lateral rays projects dorsally, forming a protuberance at the root of the postero-lateral ray, an arrangement which is found in the bursae of all the oesophagostomes of the wart hog.

*Oesophagostomum dentatum*, Rud, 1803.

Fairly common in the caecum and colon of pigs in England, and when present they are usually very numerous.

*Oesophagostomum eurycephalum*, Goodey, 1924.

Recorded by Goodey from the roan antelope and found by me in material collected from the African wart hog (*Phacocaerus aethopicus*). It has not been previously recorded from this latter host.

My description and drawings, completed before the publication of Goodey's paper, agree closely with his. As regards the external leaf crown he says it is composed of eight stout elements whose tips curve outwardly, whilst in many of my specimens the tips projected vertically out of the mouth opening. Goodey states that the prebursal papillae are absent or so small as to be undiscernible, but I found these, though small, lying at the level of the anterior end of the gubernaculum. The caudal papillae are prominent, situated about 24$\mu$ from the tip of the tail. My measurements, agreeing in the main with those of Goodey, differ in some respects. Male length 15 mm. (Goodey 9 to 10 mm.) by 0·6 mm. in breadth. Length of oesophagus 705$\mu$, posterior breadth 196$\mu$. Breadth of buccal capsule 112$\mu$, length 41$\mu$. Distance of ventral slit from anterior extremity 512$\mu$. Distance of cervical papillae from anterior extremity 426$\mu$. Spicules are 1·49 mm. in length, cross-striated and 'winged' at their distal extremities. Female 16 to 17 mm. in length (Goodey 10 to 12 mm.) by 0·5 mm. in breadth. Oesophagus 750 to 900$\mu$ in length, posterior swelling 200 to 260$\mu$ in breadth. Buccal capsule 150$\mu$ in breadth by 50$\mu$ high. Vulva 380 to 420$\mu$ from tip of tail. Vagina 160 to 180$\mu$ in length, almost transverse. Ovijector stout, 320$\mu$ in length. Anus to tip of tail 130$\mu$. Eggs 90$\mu$ by 56$\mu$.

In the male bursa the outer terminal division of the dorsal ray is as thick as, but only one-quarter the length of, the inner division.

*Oesophagostomum mwanzae*, Daubney, 1924.

From caecum and colon of wart hog, Rhodesia. It is the commonest oesophagostome of the wart hog. Lateral cephalic papillae conical and blunt; submedian elongated, with a distal rod-like portion usually bent inwards. Buccal capsule ellipsoidal in cross-section. Seen from lateral aspect the walls diverge

anteriorly ; from the dorso-ventral aspect they appear to converge anteriorly. The external leaf crown is composed of six elements, each one long and pointed, arising from base of buccal capsule. There is no internal leaf crown. The cervical groove does not extend as far as the lateral lines. The intestine at its commencement is wider than the oesophagus, pigmented, though not so deeply as in *O. dentatum* and runs a straight course to the anus. Bursa has a well-defined dorsal lobe well separated from the two lateral lobes and is longer than them. The externo-dorsal ray is bent abruptly downwards at its middle. The medio-lateral and postero-lateral rays are joined and also the ventro-ventral and latero-ventral rays. The dorsal ray was unusually stout in the specimens I examined. The outer division of each main branch is as thick as the inner, but about half its length ; gubernaculum 100 to 120$\mu$ (Goodey 160$\mu$) in length and trowel shaped. Daubney (1924) figures the handle portion as flexed dorsally, but in my specimens this part was practically straight. Seen laterally under high powers, the gubernaculum is produced at its distal extremity into a hook-like process.

The female possesses small caudal papillae, situated laterally about 30$\mu$ from the tip of the tail. My measurements differ slightly from those of Goodey. Length of male 11 to 12·2 mm. (Goodey 13 to 16 mm.), 430 to 470$\mu$ in breadth, ventral slit 280 to 295$\mu$ from anterior extremity (Goodey 0·2 mm., Daubney 0·22 mm.). Buccal capsule dorso-ventral diameter 100$\mu$ (Daubney 126$\mu$).

Female 14·7 to 18 mm. in length (Goodey 16 to 20 mm.). Anus to tip of tail 125 to 176$\mu$, vulva to tip of tail 300 to 360$\mu$. Eggs segmented when laid, 64 to 71·4$\mu$ long by 34 to 37·4$\mu$ in breadth.

Daubney, in discussing the position of *O. mwanzae* in the genus, suggests that it shows near affinities to *O. apiostomum* and *O. brumpti*. I cannot agree with this, for I regard the shape of the buccal capsule in *O. brumpti* and *O. apiostomum* (that of a rather thin-walled truncated cone) as one of the chief characters and in *O. mwanzae* the walls, seen laterally, are ellipsoidal and diverge anteriorly. The new sub-genus, *Conoweberia*, created by Ihle (1922) to include *O. apiostomum* and *O. brumpti*, indicates the importance attached by him to the shape of the buccal capsule.

Goodey considers *O. mwanzae* most nearly akin to *O. simpsoni* in the elliptical shape of the head, leaf crowns, etc., but not in the

shape of the female tail and the oesophagus. This opinion is justified, for although he was in possession of only female specimens of *O. simpsoni*, my description of the male bursa of the latter shows that it bears a marked resemblance to that of *O. mwanzae*. In *O. simpsoni* and *O. mwanzae* the dorsal lobe is longer than the lateral lobes, the externo-lateral ray is closely applied to the medio-lateral at its origin and the outer border of the main stem possesses a rounded knob at the point of origin of the postero-lateral ray. This knob exists in all the bursae of the oesophagostomes occurring in the wart hog. Of the other oesophagostomes only *O. radiatum* possesses it.

*Oesophagostomum simpsoni*, Goodey, 1924.

The material consisted of some four males and twelve females, collected from the caecum and colon of the wart hog, Rhodesia—Goodey possessed no male specimens. A rather stout species with a breadth-length ratio of 1 : 21. The body tapers gradually from the anterior two-fifths towards each extremity, the anterior extremity being truncated. The mouth collar is very flat, almost disc like, and elliptical in cross-section. The dorso-ventral diameter of the buccal capsule is the greater, being 105 to 130$\mu$, while the lateral diameter is 74 to 80$\mu$. The external leaf crown is composed of eight stout elements arising from base of buccal capsule and projecting beyond the mouth collar, with slightly divergent points. There is no internal leaf crown.

The lateral cephalic papillae are very blunt and scarcely elevate the cuticle. The submedian are elongated, inclined inwards, and have a small knob-like distal portion. The cephalic vesicle is well-developed, giving the anterior extremity, under low power magnification, an appearance not unlike the head of a match.

The cervical groove is well-defined ventrally and extends as far as the lateral lines. The cuticular inflation is continued for a short distance beyond the groove, becoming lost opposite the commencement of the intestine. There are no lateral membranes, hence the stretched-out position of the worms when fixed. The cervical papillae, similar to those of *O. dentatum*, lie opposite the posterior swelling of the oesophagus.

The oesophagus is very short and broad, scarcely enlarging

posteriorly and flattened dorso-ventrally corresponding to the shape of the mouth capsule. Its breadth-length ratio is 1 : 2·2. In *O. dentatum* it is 1 : 3·5. At the entrance to the oesophagus there is no oesophageal funnel, but at this level the oesophagus presents a short sub-globular swelling, constricts slightly at the level of the nerve ring, finally enlarging slightly and projecting for a short distance into the intestine. The intestine at its commencement is only slightly larger than the oesophagus.

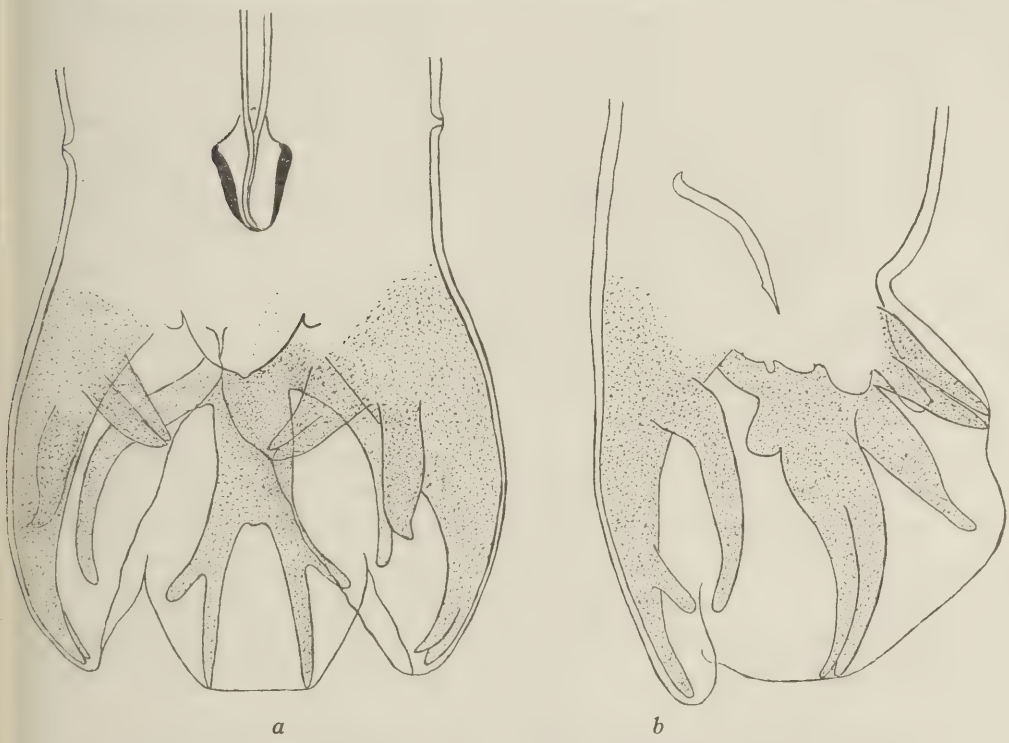


FIG. 1. *Oesophagostomum simpsoni*, Goodey. *a*.—Male bursa, ventral view ; *b*.—Same, lateral view.

The male worm has an average length of 15 mm. by 740$\mu$ broad. The oesophagus is 426$\mu$ long by 190 to 200$\mu$ broad. The ventral groove is 245$\mu$ from the anterior extremity and the cervical papillae 95$\mu$ behind this. The bursa has its dorsal lobe well separated on either side from the lateral lobes by a deep fissure, but the dorsal lobe itself is not deeply indented, though slightly longer than the lateral lobes. Prebursal papillae are present and lie opposite the anterior end of the gubernaculum. The bursal formula agrees

with that of the oesophagostomes. The ventral rays are normal and their tips reach the edge of the bursa. The externo-lateral is closely applied to the medio-lateral at its origin, then becomes well separated from it. Broader than either of the lateral rays at its origin, it becomes narrowed rather suddenly towards its extremity. The outer border of the main stem of the lateral rays, as in *O. radiatum*, *O. eurycephalum* and *O. mwanzae*, possesses a rounded knob at the point of origin of the postero-lateral ray. The lateral rays reach the edge of the bursa and their tips are strongly curved towards the dorsal rays.

The angle caused by the division of the main dorsal ray is acute; the outer terminal division of the dorsal ray is as thick as, but less than half the length of the inner terminal division and is inclined outwards. The gubernaculum is trowel-shaped, similar to that of *O. dentatum*. It is 130 to 160$\mu$ long and not markedly bent in its centre. Seen laterally the distal extremity is barbed on its dorsal aspect. The spicules are equal, alate, and appear fused at their tips.

The female worm is 15 to 21 mm. long, 0·7 to 1·05 mm. broad. The oesophagus is 430 to 460$\mu$ long by 210 to 220$\mu$ broad. The ventral slit is about 230$\mu$ from the anterior extremity, the cervical papillae 100$\mu$ behind this. Behind the genital opening the tail is straight and constricts gradually to the anus, terminating behind this in a conical point, on either side of which lies a caudal papilla. The vulva is salient and the vagina is about 150$\mu$ long and transverse. There is a double muscular ovijector about 320$\mu$ long from which two uteri run forwards. The vulva is 500 to 700$\mu$ in front of the posterior extremity, the anus 140 to 170$\mu$ from the tip of the tail.

### *Oesophagostomum oldi*, Goodey, 1924.

In examining the wart hog material, I had found and described a worm agreeing closely with Goodey's new species *O. oldi*. In the main my description and measurements agree with his, but there are several differences I wish to record.

The cephalic vesicle is but slightly developed; anterior to the cervical groove are a number of lesser circular grooves three to four in number which indent the cuticle. Goodey makes no mention of these. As the worms had been excellently preserved I cannot regard these grooves as due to shrinking of the cuticle.

Goodey notes that the external leaf crown does not arise from the base of the buccal capsule. In my specimens it takes its origin from the lower fifth of the wall.

The male measures 9·3 to 12·4 mm. in length (Goodey 11 to 13 mm.). Female 7 to 14 mm. (Goodey 15 to 18 mm.).

The tail of the female bears resemblance to the human foot, though in my specimens there is a sharp depression, not figured by Goodey, which separates the vulva and anus. Caudal papillae are prominent and slightly asymmetrical, 80 to 140$\mu$ from the tip.

The constant difference between my specimens and his lies in the length of the vagina. It is very long in both, but while Goodey gives its length as 0·8 to 1 mm., it measured in my specimens an average of 1·6 mm. and never less than 1·3 mm. The other characteristics, however, agree so closely with *O. oldi* that on these differences one is hardly justified in founding a separate species.

*Oesophagostomum yorkei*, n.sp.

Four females and two males were collected by Professor Yorke in Rhodesia, from caecum and colon of *Phacocaerus aethiopicus*.

Male about 10 mm. long by 0·4 mm. in breadth, females 14 to 15·3 mm. long by 0·65 mm. in breadth.

Mouth collar inflated, slightly elliptical in cross section. The cephalic papillae have their usual disposition, laterals blunt, submedian, long and prominent.

The cephalic vesicle is scarcely inflated. Cervical groove extends from ventral surface as far as lateral lines and is 240 to 260$\mu$ from the anterior extremity. Between the cervical groove and the head are three or four lesser grooves which indent the cuticle. The cervical papillae are like those of *O. dentatum* and are about 330$\mu$ from the anterior extremity. The mouth opening is slightly elliptical in cross section. There is an external leaf crown of eight large elements whose points project vertically beyond the mouth opening. These elements arise from the base of the inner wall of the mouth capsule. There is no internal leaf crown. The buccal capsule is about 40$\mu$ in height by 55$\mu$ in breadth. The walls in optical section appear parallel and ellipsoidal, but are constricted in their anterior fourth to form a neck. The body wall is connected by strands with the posterior aspect of the buccal capsule.

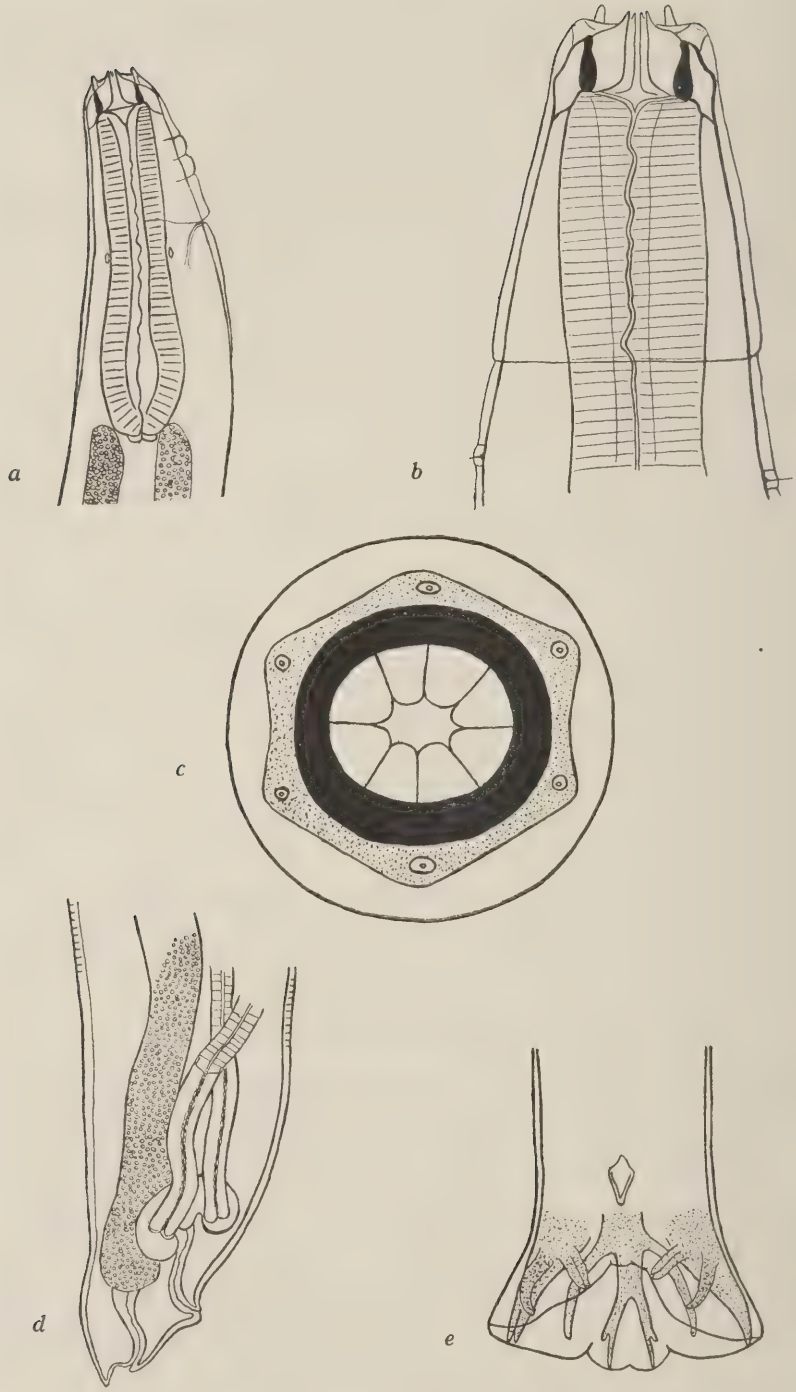


FIG. 2. *Oesophagostomum yorkei*, n.sp. *a.*—Anterior end, lateral view; *b.*—Same, ventral view, more highly magnified; *c.*—End-on view of head; *d.*—Tail end of female; *e.*—Male bursa, ventra view, somewhat flattened.

The oesophagus is about 550$\mu$ in length, of equal thickness in its anterior three fourths ; it then dilates, being here about 150$\mu$ in breadth. Oesophageal-intestinal valves small. There is an oesophageal funnel about 40$\mu$ deep lined by cuticle, but there are no teeth. The nerve ring is just behind the cervical groove.

The body is of equal width as far as the vulva, then tapers abruptly to a small, straight, conical tail. The vulva has salient lips and is about 260$\mu$ from the tip of the tail. The vagina is 190 to 210$\mu$ in length, has stout walls and is directed forwards to a double muscular ovijector about 240$\mu$ in breadth. From it two uteri run directly forwards. The rectum is about 190$\mu$ long and the anus 80$\mu$ from the tip of the tail.

*Male.* The bursa is deep and not expanded to a greater width from the rest of the body. The dorsal lobe is separated from the lateral lobes on either side by a fissure and is slightly indented in the middle line. The terminal divisions of the main dorsal ray are slender and the outer terminal division is directed straight backwards. As in *O. oldi* and *O. eurycephalum* there is a distinct rounded knob on the posterior border of the main lateral trunk at the origin of the postero-lateral ray ; externo-dorsal rays given off close to base of dorsal ray and are long and slender. Spicules are alate with their tips bent dorsally ; they are short, 1·3 mm., corresponding to shortness of vagina in female. The gubernaculum is shovel-shaped, 130$\mu$ long.

This worm appears to occupy an intermediate position as regards its relationship to the other oesophagostomes of the wart hog ; it resembles *O. oldi* in the shape of the oesophagus and the presence of a well-marked oesophageal funnel without teeth. Also in the shape of the buccal capsule and the shape and structure of the male bursa. It bears, however, an even closer resemblance to *O. eurycephalum* in the number of elements in the leaf crown and, in regard to the tail, the abrupt narrowing behind the vulva, the short almost transverse vagina and the vulva with salient lips.

This parasite has been named in honour of Professor Warrington Yorke of the Liverpool School of Tropical Medicine.

*Oesophagostomum ventri*, n.sp.

These worms, consisting of two males and fifteen females, were obtained by Dr. Thomas from the stomach of a Brazilian wild cat, together with numerous specimens of *Toxascaris marginata*. Taking into account the fact that oesophagostomes are found usually in the caecum and large colon and that available literature shows no record of the wild cat as a host of this genus, one was naturally led to the assumption that these worms were spurious parasites - the worms being found in the intestine post-mortem—owing to the cat having eaten the intestine of some other animal a short time before death.

Against this contention is the fact that the worms were in an excellent state of preservation, while the presence, in the stomach also, of specimens of *Toxascaris marginata* would serve to strengthen the evidence that these oesophagostomes were true parasites of the cat.

This worm, then, is regarded as a new species from the cat. Further evidence may show this new oesophagostome to be normally parasitic in some other animal, possibly one of the smaller rodents, which then becomes the true host.

*Description.* The worms are whitish, body practically straight, of maximum diameter at the junction of the middle and anterior fifths and diminishing gradually towards each extremity. The anterior end is truncated, the mouth collar being broad and shallow and separated from the body by a well-defined groove. There are six head papillae. The laterals are broad and flat and scarcely protrude above the surface. The sub-median are conical, not very prominent and the rather delicate pulp projecting into them from below gives each papilla in optical section an appearance not unlike the head of an arrow.

The buccal capsule is shallow, its height to breadth ratio being 1:4. The walls in section appear ' comma ' shaped, thicker anteriorly and convex on their inner aspects. From the base of the wall a chitinous ridge runs downwards and outwards to reach the body wall.

The external leaf crown arises from the anterior border of the buccal capsule. It is composed of some 45 slender pointed elements

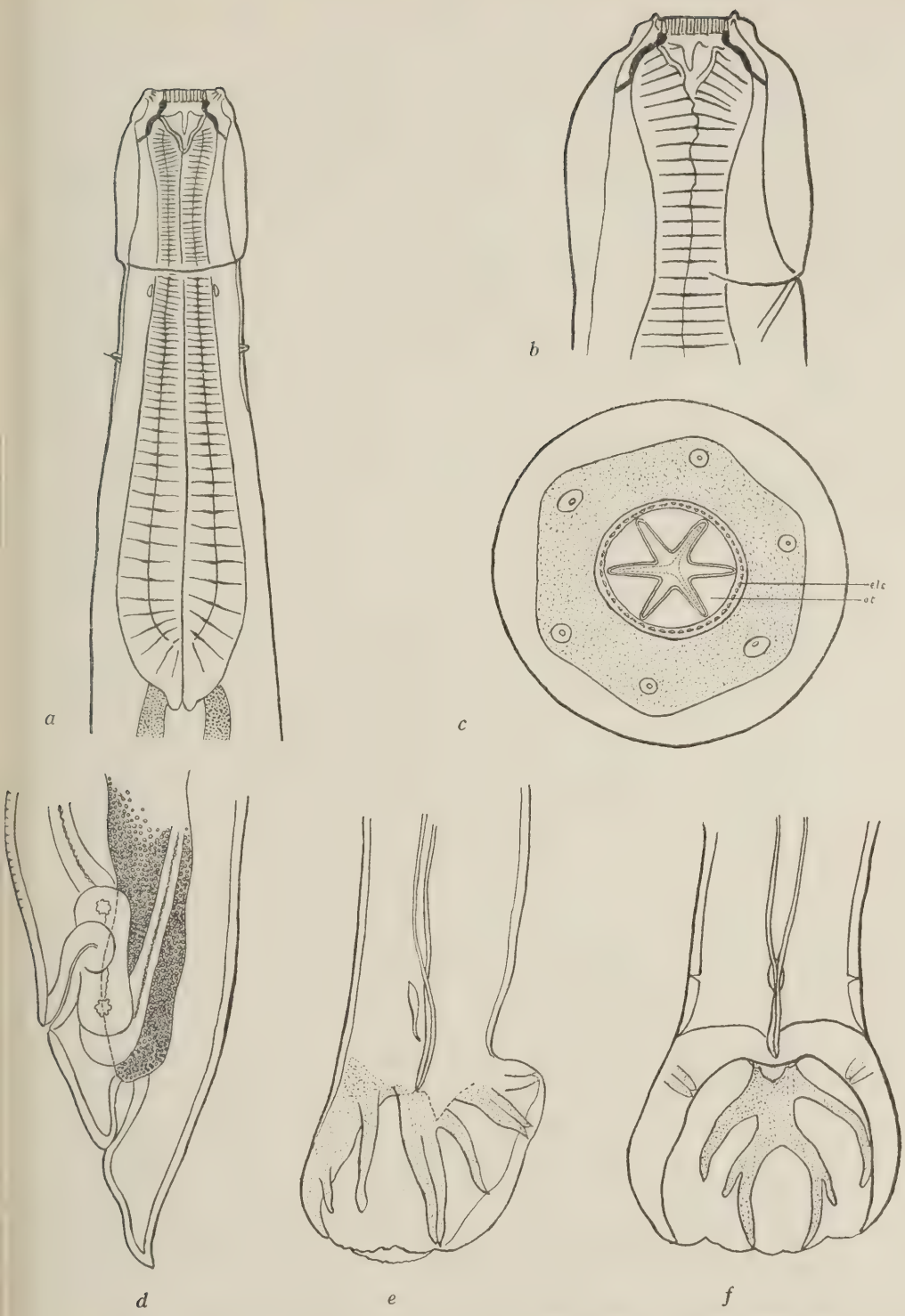


FIG. 3. *Oesophagostomum ventri*, n.sp. *a.*—Anterior end, seen ventrally; *b.*—Same, lateral view, more highly magnified; *c.*—End-on view of head; *e.l.c.*—External leaf crown, *o.t.*—oesophageal tooth; *d.*—Tail end of female; *e.*—Male bursa, lateral view; *f.*—Same, ventral view.

projecting directly forwards, but not extending beyond the mouth opening. There is no internal leaf crown. The cephalic vesicle is well-defined, constricted inferiorly by a groove extending from the ventral surface as far as the lateral lines. The excretory duct opens at this level and the nerve ring is found just behind this.

There is a large oesophageal funnel whose chitinous lining is modified to form six large teeth, their slightly acute points being directed upwards and inwards. Two teeth correspond to each oesophageal sector. The tooth of one sector is widely separated from its fellow, but lies in apposition with the tooth of the adjoining sector.

The oesophagus is long, narrows directly behind the oesophageal funnel, then gradually dilates to its posterior fifth. The cervical papillae lie just anterior to the middle of the oesophagus.

*Female.* Length 20 to 23 mm. by 670 to 700$\mu$ broad. The cuticular striations are about 17$\mu$ distant at the centre. The oesophagus is 1·23 to 1·26 mm. in length, posterior breadth about 260$\mu$. The buccal capsule is about 80$\mu$ broad by 20$\mu$ high. Distance of ventral slit from anterior extremity 330 to 360$\mu$. Cervical papillae about 540$\mu$ from anterior extremity. Caudal papillae are present on either side of the tail about 50$\mu$ from the tip. The tail is straight and slender, tapering gradually behind the vulva. The tip of the tail is sometimes bent dorsally.

The vulva is salient about 560$\mu$ from the tip. Vagina almost transverse, 190 to 260$\mu$ in length, and runs to a double muscular ovijector 200 to 270$\mu$ in length. From its extremities run two uteri, the anterior running directly forwards, the posterior running backwards, but curving almost immediately to run forwards again. The anus is non-salient, 180 to 260$\mu$ from the tip of the tail.

*Male.* About 21 mm. in length, 690$\mu$ broad. Length of oesophagus 1·23 mm., posterior breadth 230 mm. Buccal capsule 78$\mu$ broad by 19$\mu$ high. Distance from anterior extremity to ventral slit 340$\mu$, to cervical papillae 508$\mu$. The bursal formula agrees with that of the genus *Oesophagostomum*. The left externo-dorsal ray becomes detached from the main dorsal branch at about its own width above the right. The inner and outer terminal division of the dorsal ray are both slender, the former being about three times as long with slightly incurved extremities. The externo-

lateral ray is well separated from the medio-lateral throughout its length and its tip reaches nearer to the edge of the bursa than does the externo-dorsal. The spicules are equal, tubular and non-alate, with fused tips. They are 1·32 to 1·47 mm. in length. The gubernaculum, unlike that of *O. dentatum*, is lozenge-shaped, about 135$\mu$ in length. Seen laterally it is not bent markedly at its middle, but is almost straight and tapers from above. Prebursal papillae are present at the level of the gubernaculum.

In considering the relationship between this worm and the other oesophagostomes one was struck with the remarkable similarity between it and *Oesophagostomum stephanostomum* var. *Thomasi*. These latter parasites were obtained by Dr. Thomas from the intestine of a Brazilian prisoner who died in Manáos. Both are indigenous to Brazil. Railliet and Henry consider Thomas's oesophagostome, *O. dentigerum*, Railliet and Henry, 1906, from the chimpanzee and *O. stephanostomum*, Stossich, 1904, from the gorilla, are simply varieties of the one species. The only constant difference between them lies in the disposition of the terminal divisions of the posterior ray in the male bursa. With Clayton Lane I would hesitate to attach even subspecific value to this character.

Comparison with these types leads one to state that *O. ventri* is a separate species differing from *O. stephanostomum* var. *Thomasi* in the following respects :—

Table I

| | *O. stephanostomum* var. *Thomasi* | *O. ventri*, n.sp. |
|---|---|---|
| External leaf crown ... ... | 38 elements ... ... ... | 45 elements |
| Internal leaf crown ... ... | 76 small elements ... ... | None |
| Sub-median head papillae ... | Rounded, but tip produced to sharp point | Conical |
| Buccal capsule ... ... ... | Ellipsoidal in optical section ... | 'Comma'-shaped in optical section |
| Spicules ... ... ... ... | Tips free ... ... ... ... | Tips fused |
| Posterior bursal ray ... ... | Outer terminal division incurved | Outer terminal division parallel to inner |
| Oesophageal teeth ... ... | Six teeth equally spaced ... | Six teeth arranged in three pairs |

In all other respects, *e.g.*, length of spicules, position of cervical papillae, shape of tail, position of vulva and length of vagina, *O. ventri* corresponds closely with *O. stephanostomum* var. *Thomasi*.

It would seem that this carnivoran oesophagostome bears a much closer relationship to the oesophagostomes of Man and Primates than it does to the oesophagostomes of Rodentia and the Artiodactyla.

I propose for this worm the specific name *O. ventri* to mark the unusual location in the host in which it was found.

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# *SAUROSITUS AGAMAE*, N.G., N.SP. A FILARIOID PARASITE OF THE LIZARD *AGAMA COLONORUM*

BY

J. W. S. MACFIE

(*Received for publication* 19 *September*, 1924)

This worm is a common parasite of the lizard *Agama colonorum* in West Africa, the adults being found in the mesentery and the embryos in the blood. It may be noted here that the distribution of filariasis of lizards in West Africa is not uniform, for whereas two species are commonly found at Yaba, near Lagos, only one species, namely that referred to above, has hitherto been found at Sekondi (Dr. J. F. Corson), and none has yet been discovered at Accra, although the lizards there have been repeatedly examined at all seasons of the year.

### *SAUROSITUS AGAMAE*, n.g., n.sp.

Body thread-like, colourless, tapering slightly towards the posterior extremity. Anterior extremity rounded, bearing two lateral and four sub-median papillae which, however, are very small. Cuticle smooth, not striated transversely, and without either cuticular bosses, annulations, or spiral thickenings. Mouth terminal, small, slightly projecting, without lips. Oesophagus short, divided into two unequal portions, the anterior short and narrow, the posterior three or four times as long, broader, not dilated at its junction with the intestine. Nerve ring well developed. Anus sub-terminal ; tail short and blunt in both sexes.

*Male.* Shorter and more slender than the female ; length (three specimens) 42 mm. to 55 mm., breadth at the middle of the body about 200$\mu$. Posterior extremity coiled in a close, flat spiral. Cloacal opening about 15$\mu$ from the tip of the tail. Tail short and blunt ; caudal alae absent. There are four pairs of pre-anal papillae,

but no para-anal or post-anal papillae. Spicules two, sub-equal, about 100$\mu$ to 130$\mu$ in length ; gubernaculum small.

*Female.* Length (three specimens) 80 mm. to 140 mm. ; breadth at the middle of the body about 300$\mu$. Posterior extremity somewhat attenuated and terminating in two more or less distinct lobes, but without papillae. Rectum atrophied, anal orifice absent. Vulva situated just posterior to the oesophagus. Opisthodelphes. Ovoviviparous. Embryos in the blood.

*Microfilaria.* The embryos, which have been briefly described on a previous occasion (*Annals of Trop. Med. & Parasit.*, Vol. VIII, pp. 456-458, and Pl. XXV, figs. 1, 3, and 4, 1914), are enclosed in an ample sheath. The cuticle is striated. The nuclei are large, two or three abreast in the middle of the embryo, and almost completely filling the greater part of the body. The lengths of thirty specimens measured ranged from 106$\mu$ to 168$\mu$, average 135$\mu$. The breadth at the widest part is about 3$\mu$. The body does not taper towards the anterior extremity and is bluntly rounded at its end. There is usually a small area, about 4$\mu$ long, at the anterior extremity which is free from nuclei. The nerve ring is situated about 28 per cent. of the length of the body from the anterior extremity : it is a narrow, usually oblique break. The excretory pore, G1 cell, and anal pore are situated respectively 41, 74, and 88 per cent. of the length from the anterior extremity. The body tapers posteriorly to a slender tail which terminates somewhat bluntly and is occupied by nuclei to its extremity.

In addition to the embryos there occur in the blood numerous small bodies which appear in stained specimens as I-shaped structures surrounded by a cuticular envelope. These bodies are also present in the uterus of the female, and are perhaps abortive eggs.

The embryos in the blood do not exhibit periodicity and were found to be present in approximately equal numbers in blood taken from the tail at 5 a.m., 10 a.m., 5 p.m., and 10 p.m.

The distribution of the embryos in the body was studied in a lizard which had been sent to Accra from Sekondi and had died one afternoon at about 4 p.m. soon after its arrival. The tissues of this lizard were preserved and cut into sections 0·014 mm. thick, and in these sections the filarial embryos in fifty microscope fields, each 100$\mu$ square, were counted with the results shown below :—

FIG. 1. *Saurositus agamae*, n.g., n.sp. *A.*—Anterior extremity of female, lateral view. × 45. *B.*—Posterior extremity of female, ventral view. × 150. *C.*—Posterior extremity of male. × 60. *D.*—Same, lateral view. × 150. *E.*—Posterior extremity of male, ventral view to show the spicules. × *c.* 300.

TABLE I

| Tissue | Filarial larvae in 50 fields |
|---|---|
| Lung ... ... ... ... ... ... ... ... ... ... | 152 |
| Liver ... ... ... ... ... ... ... ... ... ... | 51 |
| Spleen ... ... ... ... ... ... ... ... ... | 28 |
| Blood (sinus venosus) ... ... ... ... ... ... ... | 5 |
| Heart muscle ... ... ... ... ... ... ... ... ... | 1 |
| Muscle at base of tail ... ... ... ... ... ... ... | 0 |
| Testis ... ... ... ... ... ... ... ... ... ... | 0 |

In the absence of accurate data, which are not available, as to the proportion of the various organs which is composed of blood, it is not possible to decide to what extent these figures indicate a concentration of the embryos in certain organs. With regard to the lung which is little more than a trellis for blood vessels, however, a study of the sections showed the embryos much kinked and twisted, and it was clear that such relatively large and long parasites could not flow freely through the vessels with the blood stream, and that, therefore, the lung must act as a filter, allowing the blood to pass more readily than the filarial embryos.

DIAGNOSIS. It is necessary to erect for this parasite a new genus the characters of which may be defined as follows :—Mouth without lips. Anterior extremity rounded, bearing six small papillae, two lateral and four sub-median. Cuticle smooth. Oesophagus divided into two portions.

*Male.* Posterior extremity coiled into a close spiral, tail short ; caudal alae absent, four pairs of preanal papillae ; spicules sub-equal, gubernaculum small.

*Female.* Vulva near the posterior extremity of the oesophagus ; anal orifice absent ; opisthodelphes. Microfilariae sheathed, in the blood.

HOST. Lizard, *Agama colonorum.*

LOCALITY. Nigeria and Gold Coast, West Africa.

# MISCELLANEA

## *CONTORTOSPICULUM RHEAE*

Whilst dissecting a *Rhea americana* brought to this country from South America twelve years ago, three large worms were found coiled up on the right side of the anterior abdominal air sac. The two larger worms, which were females, measured, respectively, 112·5 cms. and 101 cms., whilst the third (a male) was 20 cms. in length. No other worms were found in the air sacs, but two specimens (male and female) were embedded in the peritoneal tissue. On examination the worms were identified as *Contortospiculum rheae* (Owen, 1843), Synonym :—*Filaria horrida*, Diesing, 1851.

21 *June*, 1924.

J. Isgaer Roberts,
*Bangor University.*

## *XENOPSYLLA ASTIA*, Rothsch

*Correction of Record of its occurrence in West Africa.*

The specimens recorded as *Xenopsylla astia*, Rothsch., from Accra, Gold Coast (Evans, 1922, *Ann. Trop. Med. & Parasit.*, Vol. XVI, p. 449) have been submitted to Mr. F. S. Cox, who identified them as the closely allied *Xenopsylla nubicus*, Rothsch.

A. M. Evans.

## A CASE OF SLEEPING SICKNESS *(T. GAMBIENSE)* TREATED WITH 'BAYER 205'

H.L.S.

January, 1922. Trypanosomes found in blood in Nigeria.

May 28, 1922. Trypanosomes in blood.

Between May 30 and June 27, 1922, patient received 4 grammes of 'Bayer 205,' intravenously.

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J. W. W. Stephens.

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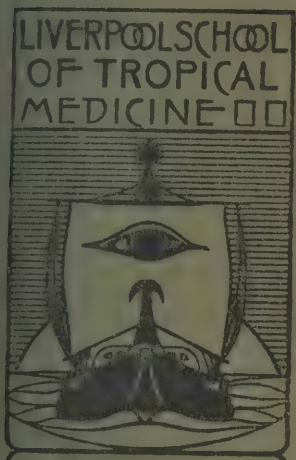


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# Annals of Tropical Medicine and Parasitology

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*1922—*

Quinton Stewart

*1923—*

John Cecil Cruickshank

*1924—*

George Maclean
Frederick John Carlyle Johnstone
Bernard Langridge Davis

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(2) A short advanced course, of one month's duration, in June.

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| 1904 | Clayton, Thomas Morrison |
| 1904 | Dalziel, John McEwen |
| 1904 | Dee, Peter |
| 1904 | Greenidge, Oliver Campbell |
| 1904 | Hehir, Patrick |
| 1904 | Khan, Saiduzzafor |
| 1904 | Laurie, Robert |
| 1904 | Maclurkin, Alfred Robert |
| 1904 | McConnell, Robert Ernest |
| 1904 | Nicholson, James Edward |
| 1904 | Philipson, Nicholas |
| 1904 | Sharman, Eric Harding |
| 1904 | Thomson, Frank Wyville |
| 1904 | Walker, George Francis Clegg |

| *Date of Diploma* | |
|---|---|
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| 1905 | Brown, Alexander |
| 1905 | Caldwell, Thomas Cathcart |
| 1905 | Critien, Attilio |
| 1905 | Hooton, Alfred |
| 1905 | Hudson, Charles Tilson |
| 1905 | Illington, Edmund Moritz |
| 1905 | Macfarlane, Robert Maxwell |
| 1905 | Maddock, Edward Cecil Gordon |
| 1905 | Moore, James Jackson |
| 1905 | Nightingale, Samuel Shore |
| 1905 | Radcliffe, Percy Alexander Hurst |
| 1905 | Young, John Cameron |
| 1906 | Adie, Joseph Rosamond |
| 1906 | Arnold, Frank Arthur |
| 1906 | Bate, John Brabant |
| 1906 | Bennetts, Harold Graves |

| *Date of Diploma* | |
|---|---|
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| 1906 | Chisholm, James Alexander |
| 1906 | Clements, Robert William |
| 1906 | Dundas, James |
| 1906 | Faichnie, Norman |
| 1906 | Jeffreys, Herbert Castelman |
| 1906 | Mackenzie, Donald Francis |
| 1906 | Pailthorpe, Mary Elizabeth |
| 1906 | Palmer, Harold Thornbury |
| 1906 | Pearse, Albert |
| 1906 | Sampey, Alexander William |
| 1906 | Smithson, Arthur Ernest |
| 1906 | Taylor, Joseph van Someron |
| 1906 | Taylor, William Irwin |
| 1906 | Tynan, Edward Joseph |
| 1906 | Watson, Cecil Francis |
| 1906 | Willcocks, Roger Durant |
| 1906 | Williamson, George Alexander |
| 1907 | Allan, Alexander Smith |
| 1907 | Allwood, James Aldred |
| 1907 | Bond, Ashton |
| 1907 | Branch, Stanley |
| 1907 | Collinson, Walter Julius |
| 1907 | Davey, John Bernard |
| 1907 | Donaldson, Anson Scott |
| 1907 | Fell, Matthew Henry Gregson |
| 1907 | Gann, Thomas William Francis |
| 1907 | Graham, James Drummond |
| 1907 | Hiscock, Robert Carroll |
| 1907 | Keane, Joseph Gerald |
| 1907 | Kennan, Richard Henry |
| 1907 | Kenrick, William Hamilton |
| 1907 | Le Fanu, George Ernest Hugh |
| 1907 | Mackey, Charles |
| 1907 | Maddox, Ralph Henry |
| 1907 | McCarthy, John McDonald |
| 1907 | Raikes, Cuthbert Taunton |
| 1907 | Ryan, Joseph Charles |
| 1907 | Vallance, Hugh |
| 1908 | Caverhill, Austin Mack |
| 1908 | Crawford, Gilbert Stewart |
| 1908 | Dalal, Kaikhusroo Rustomji |
| 1908 | Dansey-Browning, George |
| 1908 | Davidson, James |
| 1908 | Dickson, John Rhodes |
| 1908 | Dowdall, Arthur Melville |
| 1908 | Glover, Henry Joseph |
| 1908 | Greaves, Francis Wood |
| 1908 | Goodbody, Cecil Maurice |
| 1908 | Harrison, James Herbert Hugh |
| 1908 | Joshi, Lemuel Lucas |
| 1908 | Le Fanu, Cecil Vivian |
| 1908 | Luethgen, Carl Wilhelm Ludwig |
| 1908 | Mama, Jamshed Byramji |
| 1908 | McCay, Frederick William |
| 1908 | McLellan, Samuel Wilson |
| 1908 | Pearce, Charles Ross |
| 1908 | Schoorel, Alexander Frederik |
| 1908 | Smith, John Macgregor |
| 1908 | Stewart, George Edward |
| 1908 | Tate, Gerald William |
| 1908 | Whyte, Robert |

| *Date of Diploma* | |
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| 1909 | Allin, John Richard Percy |
| 1909 | Armstrong, Edward Randolph |
| 1909 | Barrow, Harold Percy Waller |
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| 1909 | Carr-White, Percy |
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| 1909 | Cope, Ricardo |
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| 1909 | Innes, Francis Alexander |
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| 1909 | McCabe-Dallas, Alfred Alexander Donald |
| 1909 | Meldrum, William Percy |
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| 1909 | Samuel, Mysore Gnananandaraju |
| 1909 | Shroff, Kawasjee Byramjee |
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| 1909 | Turkhud, Violet Ackroyd |
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All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

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# POLYMORPHIC TRYPANOSOMES OF THE *T. BRUCEI* GROUP RECOVERED FROM THE MWANZA SLEEPING SICKNESS AREA

BY

H. LYNDHURST DUKE, O.B.E., M.D., Sc.D. (Cantab.),

BACTERIOLOGIST, UGANDA PROTECTORATE.

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## INTRODUCTION

The trypanosome strains dealt with in this paper were recovered from the Sleeping Sickness area in the neighbourhood of Mwanza in Tanganyika Territory.

During a tour through the fly-belt in July and August, 1922, I isolated four different strains, all of which were inoculated into experimental monkeys. The strains included two from man,

one from wild fly, and one from wild game. The monkey carrying the wild game strain escaped; the other strains reached the Laboratory at Entebbe, and form the subject of the studies here set forth.

Several papers have been published dealing with the Mwanza outbreak of the human trypanosomiasis (Swynnerton, 1923 a and b; Duke, 1923a). The disease in man was generally acute and fatal, and, in places, attained epidemic proportions. The causative trypanosome, when inoculated into guinea-pigs, showed posterior-nuclear forms, and was accordingly diagnosed as of the nature of *T. rhodesiense*, rather than *T. gambiense*. The outbreak presented many points of interest. The obscurity surrounding the origin of the human parasite; its apparently sudden appearance, and its mode of propagation; the occurrence of a *T. rhodesiense* like organism in epidemic form, and so far north; the discovery that the insect vector was a new species, *G. swynnertoni*—all these features combined to produce a novel and fascinating problem.

The first part of the present paper deals with the three strains during their sojourn at the Entebbe Laboratory. They have been studied in relation to both the mammalian and the insect host. Incidentally, in the course of these studies, further information has been obtained about the behaviour of trypanosome strains when subjected to direct transmission over a prolonged period.

Part II represents an attempt to assemble and review the data hitherto obtained by the experimental study of directly-transmitted strains, and to set forth the conclusions resulting from these studies.

## PART I

### A.—ACCOUNT OF THE MAINTENANCE OF THE STRAINS AND THEIR ANIMAL REACTIONS

(1) Human Strain, 483.

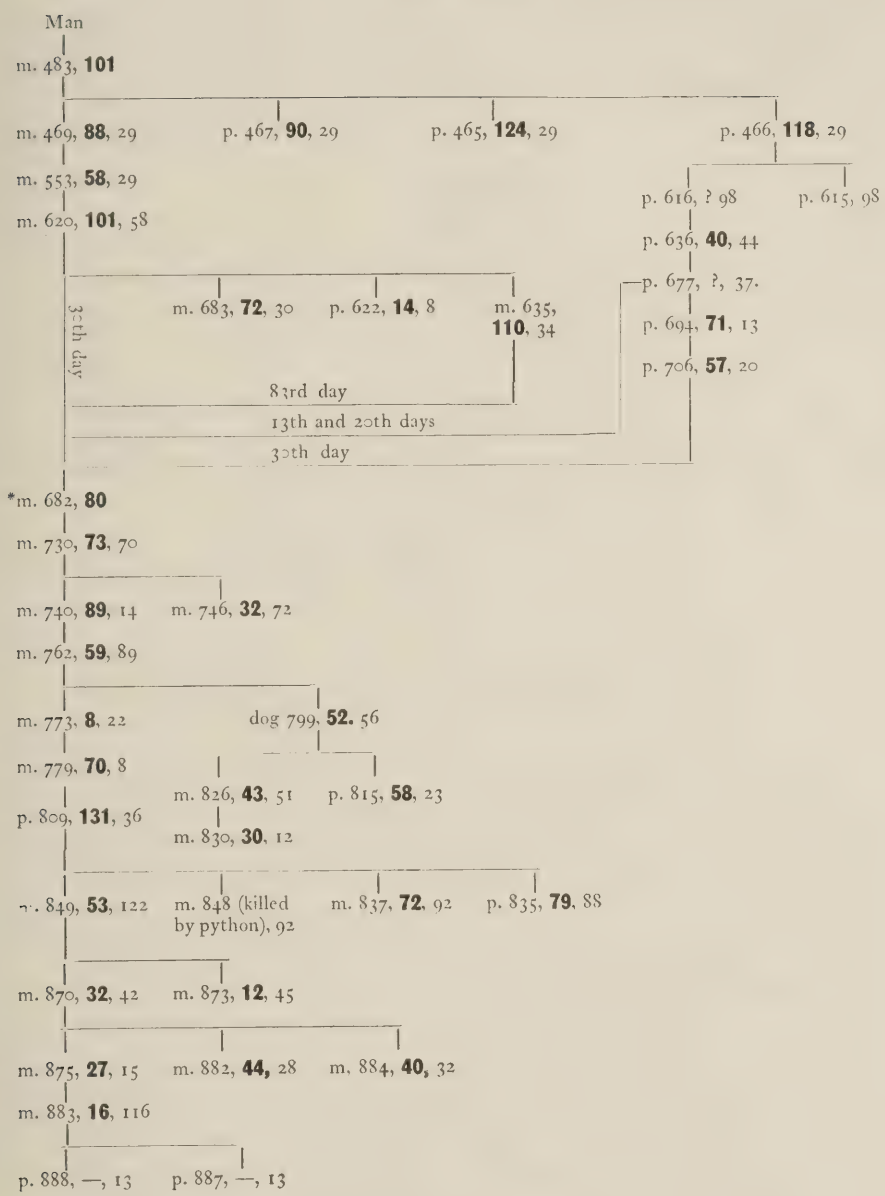
Inoculated on 1.8.22 from young adult native S. of Nyamagana village in the fly area.

The upkeep of this strain is shown in Table I.

The figures in black type give the duration of the disease in the animal; the other figure gives the day of the disease in the infecting animal when the sub-inoculation was made.

TABLE I.

Human Strain 483.




* Monkey 682 received in all 5 inoculations, as shown in the Table.

NOTE. In this and other similar Tables m. signifies monkey, and p. guinea-pig.

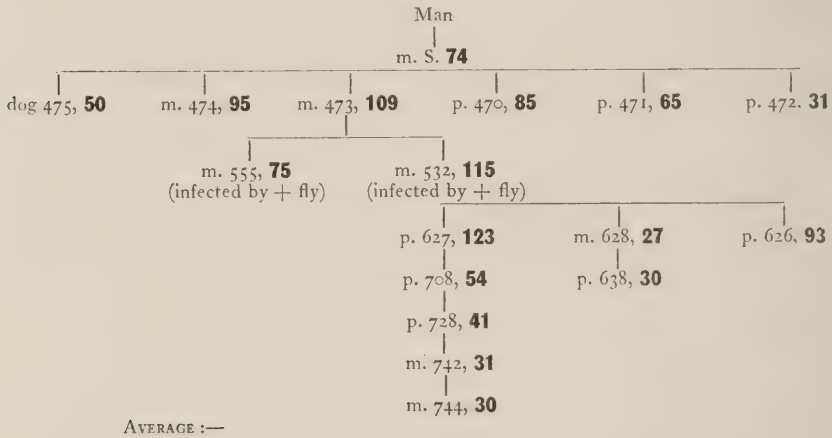
(2) Human Strain S.

Monkey S. was inoculated on 2.8.22 from an adult male native M., of Gamboshi village in the fly-belt.

Table II shows the upkeep of this strain until July, 1923, when circumstances necessitated its abandonment.

Table II.

Human Strain S.




Average :—

| | |
|---|---|
| 8 completed monkeys ... ... ... ... ... | 69.5 days. |
| 8 completed guinea-pigs ... ... ... ... ... | 65.2 days. |
| 1 dog ... ... ... ... ... ... ... | 50 days. |

A young chimpanzee was fed upon by infective flies in Experiments 527 and 530 (of Tables IV and V), on two consecutive days. Trypanosomes were seen in its blood six days later, and it died sixty days after infection. This chimpanzee seemed quite well up to the day when its companion died (*cf.* below) ; from then on it began to mope and refuse its food. It is difficult to decide to what extent grief influenced the course of the infection.

Both the natives from whom these two human strains were derived denied knowledge that they were sick ; they were detected at the inspection musters held at villages in the fly-belt. They both had enlarged cervical and axillary glands, and looked as though they had recently lost flesh. The disease was, to all appearances, not very advanced. The Senior Commissioner, Mwanza, informs me that native S. died on 12.2.23, and native M. during December, 1922.

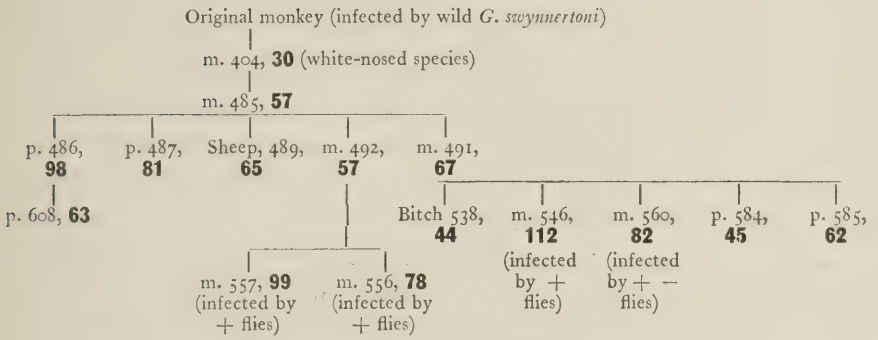
(3) Wild Fly Strain, E.

Recovered by feeding wild *G. swynnertoni* on a clean experimental monkey. Trypanosomes appeared first in the monkey's blood on 26.7.22.

Table III shows the maintenance of this strain, which was relinquished in January, 1923.

Table III.

Wild Fly Strain E.




Average :—

| | |
|---|---|
| 7 monkeys ... ... ... ... ... ... ... | 78.8 days. |
| 5 guinea-pigs ... ... ... ... ... ... ... | 69.8 days. |
| 1 bitch ... ... ... ... ... ... ... ... | 44 days. |
| 1 sheep (native) ... ... ... ... ... ... .. | 65 days. |

An old chimpanzee, fed upon by flies (Experiment 531, Tables VIII and IX), on a single occasion, became infected and died forty-eight days later. Four infective flies fed on this animal, and trypanosomes were already numerous in the peripheral blood on the ninth day after infection.

It is plain from Tables I, II and III that the three strains, when first isolated, were essentially identical in their behaviour in laboratory animals. Nor is any difference discernible in the morphology of the strains.

(4) Posterior-nuclear Forms :—

All these strains have been examined at intervals for posterior-nuclear forms, two hundred trypanosomes being inspected in each of a series of stained thin blood-films from infected guinea-pigs. In all the strains these forms were found, though latterly not at

every examination. In some guinea-pigs they were less common than in others. In guinea-pig 809 of Table I they were generally difficult to find. In the case of strain 483, posterior-nuclear forms are apparently less common now than formerly ; for example, they were always common in guinea-pig 835 of Table I, but rarer and often of less pronounced type in guinea-pigs 887 and 888.

Thus the Mwanza human strain 483 can still produce posterior-nuclear forms, though it has lost the power of cyclical development in tsetse ; it is, however, not quite clear whether the two processes are entirely independent. Now it is interesting to note that both the Direct Transmission Strain and the Antelope Strain ceased to produce posterior-nuclear forms in guinea-pigs in the later stages of the upkeep of these strains at the laboratory. It has been suggested elsewhere (Duke, 1921) that these two strains are descendants of trypanosomes which, at the time of the great epidemic in Uganda, were capable of infecting man, and that they have assumed certain of the characteristics of *T. brucei* as the result of long-continued and intimate association with *Tragelaphus spekei*. On the other hand, the Mwanza human trypanosomes are indistinguishable by laboratory tests from *T. brucei*. It is tempting to see, in the observations just recorded, confirmation of these views on the origin of the Uganda and Tanganyika strains, respectively. The power exhibited by the two former of producing posterior-nuclear forms, being a comparatively recent acquisition, is soon lost ; whereas, in the case of the Mwanza strain, the characters of typical *T. brucei* are more deeply rooted, and so persist longer under radical changes of environment.

These studies also show that increase of the virulence of a strain of trypanosomes is not necessarily associated with the formation by the strain of posterior-nuclear forms in suitable animals.

## B.—ACCOUNT OF TRANSMISSION EXPERIMENTS CARRIED OUT WITH LABORATORY-BRED *G. PALPALIS*

### (1) Human Strain 483.

Table IV sets forth transmission experiments carried out with this strain shortly after its arrival at the laboratory, and Table V gives the results obtained by dissecting the positive flies obtained in these experiments.

Table IV.

| Experiment No. | Infecting monkey | Date | Flies dissected | | | Day on which dissection began | Alive 25th day | Result of feeding on clean monkey | Date at which box became infective | Flies containing flagellates | Duration of experiment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Males | Females | Total | | | | | | |
| 503 | 468 | 11–13.9.22 | 22 | 23 | 45 | 12 | 42 | + | 26 | 1 | 38 |
| 527 | 469 | 21–23.9.22 | 26 | 34 | 60 | 8 | 54 | + | 18 | 9 | 36 |
| 530 | 468 | 23–25.9.22 | 35 | 32 | 67 | 7 | 59 | + | | 7 | 34 |
| 539 | 469 | 26–28.9.22 | 37 | 31 | 68 | 11 | 66 | + | 20 | 3 | 32 |
| *583 | 469 | 11–12.11.22 | 23 | 35 | 58 | 16 | 54 | — | — | 2 | 66 |
| *586 | 469 | 12–13.11.22 | 26 | 31 | 57 | 16 | 53 | — | — | 6 | 66 |
| *588 | 469 | 13–14.11.22 | 35 | 31 | 66 | 23 | 63 | — | — | 3 | 64 |

The brackets indicate that the two boxes were fed upon the same clean monkey on alternate days.

Note.—The * indicates that the flies in these three boxes were fed throughout the experiment upon a baboon, an animal not susceptible to this strain of trypanosomes.

TABLE V.

| Experiment No. | No. of fly containing flagellates | Day of experiment on which fly was dissected | Intensity of infection | | Sex of fly |
|---|---|---|---|---|---|
| | | | Gut | Glands | |
| 503 | 1 | 38 | +++ | +++ | Male |
| 527 | 2 | 27 | +++ | + | Female |
| — | 3 | 32 | +++ | +++ | Male |
| — | 4 | 32 | +++ | +++ | — |
| — | 5 | 33 | +++ | ++ | — |
| — | 6 | 36 | +++ | +++ | — |
| — | 7 | 36 | +++ | +++ | Female |
| — | 8 | 36 | +++ | +++ | — |
| — | 9 | 36 | +++ | +++ | — |
| — | 10 | 36 | +++ | — | — |
| 530 | 11 | 7 | ++ | — | — |
| — | 12 | 7 | ++ | — | — |
| — | 13 | 34 | +++ | +++ | Male |
| — | 14 | 34 | +++ | +++ | — |
| — | 15 | 34 | +++ | +++ | — |
| — | 16 | 34 | +++ | +++ | Female |
| — | 17 | 34 | +++ | +++ | — |
| 539 | 18 | 32 | +++ | +++ | Male |
| — | 19 | 32 | +++ | +++ | Female |
| — | 20 | 32 | +++ | +++ | — |
| 583 | 21 | 15 | +++ | — | Male |
| — | 22 | 40 | +++ | — | — |
| 586 | 23 | 41 | + | — | — |
| — | 24 | 48 | + | — | — |
| — | 25 | 48 | + | — | — |
| — | 26 | 66 | +++ | +++ | — |
| — | 27 | 66 | +++ | +++ | Female |
| — | 28 | 66 | ++ | — | — |
| 588 | 29 | 39 | +++ | ++ | Male |
| — | 30 | 44 | +++ | +++ | — |
| — | 31 | 62 | +++ | +++ | — |

(2) Human Strain S.

Table VI gives the transmission experiments with this strain, and Table VII the results of dissecting the positive flies obtained.

Table VI.

| Experiment No. | Infecting monkey | Date | Flies dissected | | | Day on which dissection began | Alive 25th day | Result of feeding on clean monkey | Date at which box became infective | Flies containing flagellates | Duration of experiment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Males | Females | Total | | | | | | |
| 521 | 473 | 19–21.9.23 | 31 | 28 | 59 | 10 | 46 | + | 25 | 2 | 32 |
| 528 | 473 | 22–24.9.23 | 30 | 31 | 61 | 10 | 44 | + | 19 | 7 | 29 |

Table VII.

| Experiment No. | No. of flies containing flagellates | Day of experiment on which fly was dissected | Intensity of infection | | Sex of fly |
|---|---|---|---|---|---|
| | | | Gut | Glands | |
| 527 | 1 | 32 | +++ | +++ | Male |
| — | 2 | 32 | +++ | +++ | — |
| 528 | 3 | 20 | +++ | +++ | Female |
| — | 4 | 28 | +++ | +++ | Male |
| — | 5 | 28 | +++ | +++ | — |
| — | 6 | 28 | +++ | +++ | — |
| — | 7 | 28 | +++ | — | Female |
| — | 8 | 29 | +++ | +++ | Male |
| — | 9 | 29 | +++ | +++ | — |

(3) Wild Fly Strain E.

Table VIII gives the transmission experiments, Table IX the results of the fly dissections with this strain.

Table VIII.

| Experiment No. | Infecting monkey | Date | Flies dissected | | | Day on which dissection began | Alive 25th day | Date at which box became infective | Flies containing flagellates | Result of feeding on clean monkey | Duration of experiment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Males | Females | Total | | | | | | |
| 513 | 491 | 16, 17, 18.9.22 | 21 | 19 | 40 | 12 | 34 | — | 0 | — | 44 |
| 526 | 491 | 21–23.9.22 | 32 | 39 | 71 | 8 | 62 | 22 | 23 | + | 35 |
| 531 | 492 | 23–25.9.22 | 41 | 23 | 64 | 9 | 54 | | 7 | | 33 |
| 540 | 491 | 26–28.9.22 | 40 | 18 | 58 | 25 | 56 | 23 | 3 | + | 34 |
| 542 | 492 | 27–29.9.22 | 30 | 43 | 73 | 8 | 55 | | 5 | | 34 |
| 544 | 489 | 28, 29, 30.9 and 1 and 3.10.22 | 28 | 23 | 51 | 7 | 44 | 22 | 4 | + | 34 |
| 545 | 489 | 28.9–1.10.22 | 34 | 29 | 63 | 8 | 53 | | 0 | | 35 |
| 547 | 492 | 30.9–2.10.22 | 22 | 26 | 48 | 8 | 37 | 26 | 1 | | 32 |
| 548 | 491 | 2.10–4.10.22 | 32 | 35 | 67 | 6 | 52 | | 3 | + | 29 |

The brackets indicate that the two boxes were fed upon the same clean monkey on alternate days.

Table IX.

| Experiment No. | No. of fly containing flagellates | Day of experiment on which fly was dissected | Intensity of infection | | Sex of fly |
|---|---|---|---|---|---|
| | | | Gut | Glands | |
| 526 | 1 | 10 | +++ | (not seen) | Female |
| — | 2 | 35 | ++ | — | Male |
| 531 | 3 | 14 | +++ | — | Female |
| — | 4 | 18 | +++ | — | — |
| — | 5 | 33 | +++ | — | Male |
| — | 6 | 33 | +++ | +++ | Female |
| — | 7 | 33 | +++ | +++ | Male |
| — | 8 | 33 | +++ | +++ | — |
| — | 9 | 33 | +++ | +++ | — |
| 540 | 10 | 33 | +++ | +++ | — |
| — | 11 | 32 | +++ | +++ | — |
| — | 12 | 32 | +++ | +++ | Female |
| 542 | 13 | 17 | +++ | — | Male |
| — | 14 | 20 | +++ | — | Female |
| — | 15 | 34 | +++ | +++ | — |
| — | 16 | 34 | +++ | +++ | — |
| — | 17 | 34 | +++ | +++ | — |
| 544 | 18 | 20 | — | — | Male |
| — | 19 | 33 | +++ | — | — |
| — | 20 | 34 | +++ | +++ | — |
| — | 21 | 34 | — | — | — |
| 547 | 22 | 20 | +++ | — | Female |
| 548 | 23 | 26 | +++ | + | Male |
| — | 24 | 29 | +++ | + | — |
| — | 25 | 29 | +++ | + | Female |

It will be seen from Tables IV-IX that all the three strains were readily transmissible by *G. palpalis* at the time of their isolation from the fly-belt.

The experiments arrayed in Table X were carried out in March and April, 1924, with the only strain surviving at that time, Human Strain 483.

TABLE X.

| Experiment No. | Infecting monkey | Day on which dissection of flies began | No. of flies alive on 20th day | Flies dissected | | | Duration of experiment days | Day of disease in infecting monkey when flies put on |
|---|---|---|---|---|---|---|---|---|
| | | | | Males | Females | Total | | |
| 1 | 837 | 14 | 45 | 27 | 22 | 49 | 31 | 36-38 |
| 2 | 848 | 29 | 50 | 24 | 26 | 50 | 31 | 7-9 |
| 3 | 837 | 9 | 47 | 24 | 27 | 51 | 30 | 39-41 |
| 4 | 848 | 11 | 55 | 29 | 30 | 59 | 32 | 10-12 |
| 5 | 849 | 22 | 62 | 33 | 29 | 62 | 32 | 11-13 |
| 6 | 837 | 9 | 65 | 42 | 30 | 72 | 33 | 42-44 |
| 7 | 848 | 8 | 50 | 25 | 29 | 54 | 33 | 13-15 |
| 8 | 849 | 9 | 68 | 35 | 34 | 69 | 36 | 14-16 |
| 9 | 837 | 8 | 43 | 44 | 12 | 56 | 37 | 45-47 |
| 10 | 849 | 11 | 54 | 30 | 29 | 59 | 36 | 17-19 |
| 11 | 848 | 10 | 51 | 38 | 23 | 61 | 39 | 17-19 |
| 12 | 837 | 6 | 56 | 36 | 27 | 63 | 38 | 49-51 |
| 13 | 848 | 8 | 57 | 34 | 33 | 67 | 38 | 20-22 |
| 14 | 849 | 9 | 41 | 33 | 26 | 59 | 38 | 21-23 |
| 15 | 837 | 8 | 32 | 25 | 27 | 52 | 42 | 52-54 |
| 16 | 848 | 11 | 57 | 34 | 27 | 61 | 41 | 23-25 |
| 17 | 837 | 11 | 47 | 36 | 23 | 59 | 35 | 55-57 |
| 18 | 848 | 9 | 56 | 38 | 29 | 67 | 35 | 26-28 |
| 19 | 849 | 8 | 47 | 26 | 33 | 59 | 37 | 28-31 |
| 20 | 837 | 11 | 56 | 34 | 26 | 60 | 38 | 58-60 |
| 21 | 837 | 9 | 50 | 36 | 19 | 55 | 36 | 61-63 |
| 22 | 849 | 9 | 30 | 24 | 22 | 46 | 37 | 31-33 |
| 23 | 849 | 10 | 58 | 37 | 30 | 67 | 38 | 32-34 |
| 24 | 837 | 10 | 60 | 29 | 34 | 63 | 38 | 63-65 |
| 25 | 849 | 12 | 31 | 15 | 19 | 34 | 37 | 35-37 |
| 26 | 837 | 9 | 19 | 12 | 16 | 28 | 38 | 65-67 |
| | Totals | ... ... | 1287 | 800 | 682 | 1482 | | |

Of the 1,482 flies dissected during these experiments, six were found to contain flagellates. In every case, however, the organisms were exceedingly scarce, amounting to a few individuals in the whole preparation of the teased-out gut. The particulars of these six flies are as follows :—

1. *Experiment* 2 : One female killed on thirty-first day after first infective feed.
2. *Experiment* 6 : One eleventh day male.
3. *Experiment* 8 : One ninth day male.

4 and 5. *Experiment* 9 : One tenth day male, and one thirty-fifth day male.

6. *Experiment* 10 : One twenty-second day male.

The minute infections shown by these flies were very remarkable. They were only revealed by a careful search of the freshly-teased gut, and sometimes only two or three flagellates were seen. They must be regarded as abortive attempts at development by a strain which has all but completely lost its power of maintaining itself in the alimentary canal of *Glossina*. When a normal fly-strain is exposed to tsetse the trypanosomes die out at once in the majority of the flies ; but, in the favourable minority, development proceeds uninterruptedly, until the gut of the insect is swarming with flagellates.

The condition in these six flies is quite different from anything hitherto observed, and represents a still further stage in the degeneration process already demonstrated in earlier work done with the Antelope Strain (Duke, 1923b).

## PART II

### A.—RESUMÉ OF EXPERIMENTAL WORK ON DIRECT TRANSMISSION OF TRYPANOSOMES

The experiments set forth in this paper complete a series of three parallel studies on the effect of continued direct transmission on polymorphic trypanosomes of the *brucei* group. The strains examined are the Direct Transmission Strain, isolated from wild Lake-shore *G. palpalis* in January, 1920; the Antelope Strain, isolated from a situtunga antelope on Damba Island in September, 1920; and the Mwanza Human Strain, 483 (Duke 1921, 1923a and 1924).

All these strains, when first isolated, were readily transmissible cyclically by laboratory-bred *G. palpalis* at the Entebbe Laboratory. A comparison of the histories of the three strains since their arrival at the Laboratory is interesting.

(1) Direct Transmission Strain.

After 11 direct passages, covering 16 months, was still cyclically transmissible by tsetse.

After 23 more passages, over a further period of 14 months, was no longer able to develop at all in tsetse. The exact period at which this change set in was not determined. A sudden increase of virulence appeared about the eighteenth passage, after 23 months' maintenance by direct transmission. This increase in virulence apparently occurred at about the time that the trypanosomes lost their power of developing in tsetse.

(2) Antelope Strain.

After 24 direct passages, over a period of about 22 months, had lost the power of invading the salivary glands of the fly; heavy gut-infections were still obtained, but here also there were signs of impairment in developing power. Ten months later, after 20 more passages, all power of development in tsetse had disappeared. The exact date at which this last stage was reached was not determined.

The virulence of this strain, in monkeys, apparently began to increase about the seventeenth direct passage, *i.e.* after about

15 months of maintenance. The average duration of the disease in the 24 monkeys employed during the first 16 direct passages was 34 days; during the subsequent 34 passages, extending over a period of 20 months, the average duration for 20 monkeys was 24 days.

It is impossible to determine with certainty if the virulence began to increase coincidently with the onset of impairment in the power of cyclical development in the fly. By the time the trypanosome had lost its hold on the salivary glands of the fly, virulence had begun to increase, and it may well be that the two processes were intimately associated.

(3) Mwanza Human Strain.

Table V suggests that the strain had already undergone slight modification in relation to tsetse towards the end of its sojourn in monkey 469, *i.e.* during the second direct passage from man, and only two months after isolation. This inference is, however, by no means certain, as the baboon's blood may have influenced the development of the flagellates. The passage to the next monkey of the series was made by flies cyclically infected from 469. Eleven months later, after eight direct passages, the strain had all but completely lost the power of development in tsetse. The virulence of this strain in monkeys showed no increase at the time that the last transmission experiments (shown in Table X) were carried out. But in the passages immediately following, an increase was at length manifest, and the strain has been now transferred to guinea-pigs.

It will be noted that the Mwanza Strain lost the power of cyclical development in tsetse much more rapidly than did the two Uganda strains. Both the latter were undoubtedly cyclically transmitted previous to their isolation in monkeys. On the other hand, there are reasons for suspecting that Strain 483 may have been subjected to a number of direct passages from man to man in the fly-belt before reaching native S, and this may explain the relatively rapid alteration of this strain after its arrival at the laboratory.

B.—DISCUSSION

The results obtained from these transmission experiments, though of scientific interest, are not necessarily valid for human trypanosomiasis. Monkeys cannot be regarded as true natural hosts of the trypanosome ; and propagation by the syringe differs in many respects from any natural method of transmission. We are, indeed, witnessing the behaviour of what is practically a *monkey strain* of a trypanosome under peculiar conditions of maintenance, and we have recorded the appearance of certain alterations in the physiology of the strain. But caution is necessary in attempting to assign a cause for these changes, lest we ascribe to the agency of direct transmission effects due wholly, or partially, to the tissue reactions of the *Cercopithecus*. Not that there is any reason to suppose that the host factor alone, without the co-operation of direct transmission, can account for the loss of the power of a strain of trypanosomes to develop cyclically in tsetse. Provided that the passage of the monkey-strain from animal to animal is always effected by cyclically infected flies, it is a reasonable, though unproven, assumption that the parasite will retain indefinitely its power to infect the salivary glands of the fly. Presumably, too, any departure from the cyclical method will tend, sooner or later, to alter the constitution of the trypanosome ; though in the case of the Direct Transmission Strain (Duke 1921), the organism was still capable of full cyclical development in *Glossina* after eleven monkey-passages by the direct method over a period of some nine months.

On biological grounds we should expect that cyclical transmission, acting over a prolonged period, would lead to a gradual adjustment of the trypanosome to its mammalian host. An excellent example of this is the equilibrium established between ruminant game and *T. brucei*. In nature, this adjustment will be found to exist between a trypanosome and those species of animals upon which it is normally dependent. In certain *palpalis* regions there is evidence that a balance has been established between man and a member of the polymorphic group of trypanosomes. But in most, if not all, human communities where trypanosomiasis occurs, man is evidently too susceptible to qualify as an ideal host ; and while we have no evidence that game animals in their wild state are inconvenienced by their trypanosomes, in the case of man the difficulty is rather to prove the existence of any genuine tolerance.

Vertebrates in which the trypanosome provokes a rapidly fatal disease cannot be regarded as biologically perfect hosts of the parasite. Monkeys fall into this category, and so, to a great extent, does man. The strains which we have been studying in monkeys were maintained in an artificial environment. They destroyed their hosts rapidly ; could not develop in tsetse ; and were absolutely dependent on direct transmission for survival. The Mwanza human trypanosomes were, as a rule, rapidly fatal to their human hosts, though retaining (at the time of the first sub-inoculations into monkeys) the power to survive in tsetse ; and there are reasons for believing that direct transmission played an important part in the spread of the Mwanza disease in man. To a certain extent, therefore, the monkey-strains under review are comparable with the virulent human strains in the fly-belt, *i.e.* they are both utilising vertebrate hosts to which they are imperfectly adjusted biologically. The closeness of the parallel will, of course, depend upon the extent to which vertebrates other than man are accessible to the trypanosome ; in other words, to the frequency with which direct transmission from man to man is effected. In all probability, therefore, similar causes will produce similar effects, and the evolution of these virulent human strains in the fly-belt, if left to nature, will be subject to the laws that govern the behaviour of our monkey strains at the laboratory.

The *brucei-gambiense* group of trypanosomes is widely associated in nature with certain species of ruminant game. The organisms are transmitted by cyclical passage through the *Glossina* that feed upon these animals, and, apparently, no ill-effects are caused to the mammalian host. When circumstances compel a trypanosome to resort to species of mammals that possess little or no tolerance towards it, cyclical transmission by *Glossina* will tend to establish equilibrium between the parasite and its vertebrate host. If the mammal be unable to tolerate the infection and equilibrium is unattainable, the trypanosome may gradually lose its power of cyclical development, until it becomes entirely dependent on direct transmission for its propagation. For a time, however, even in highly susceptible hosts, cyclical transmission will still function, and will, no doubt, tend to delay the appearance in the strains so transmitted of the fulminant characters so favourable to the direct method.

We know of the existence in nature of three free-flagellated mammalian trypanosomes which have dispensed with cyclical

transmission—*T. evansi*, *T. equinum* and *T. equiperdum*. As far as is known, all these organisms rely for their propagation entirely on direct transference from host to host. Surra, mal de caderas and dourine are generally recognisable by the clinical symptoms they produce. But apart from the recognition of these organisms on clinical or morphological grounds, it is a somewhat tedious process to demonstrate the inability of a trypanosome to develop cyclically in *Glossina*, and this test is not often applied to strains isolated in the field. I do not know whether posterior-nuclear forms occur in animals infected with surra; but, in any case, the recovery from game or stock of a virulent trypanosome of the *brucei* group, incapable of cyclical development in tsetse, would inevitably suggest thoughts of *T. evansi*. It may well be that these three trypanosomes have evolved, in the manner outlined above, from fully-equipped tsetse strains of the *brucei* type.

Unfortunately for our present investigation, man is distinguished among the mammals commonly fed upon by *Glossina*, in that his relation to the trypanosomes of the *brucei-gambiense* group ranges from complete immunity to acute susceptibility. The baboon, which is exceedingly resistant to all trypanosomes, has never been found naturally infected, although it has, on rare occasions, been infected in the laboratory. This animal cannot, therefore, be made to take the place of man in experimental investigations of the polymorphic group. As far as is known, the chimpanzee is susceptible to all the trypanosomes of this group. It is man's unique position with regard to trypanosomes that makes the problems of human trypanosomiasis so difficult to solve. In *palpalis* areas, the conclusions resulting from our laboratory investigations of monkey-strains can be applied intelligibly to the study of the trypanosomes in man. Both endemic and epidemic types of the human disease are known to occur, and it is conceivable that direct transmission strains, incapable of cyclical development in tsetse, do actually exist in certain human communities. But in game-tsetse areas the case is different and more difficult to unravel. Man is very much more resistant to the trypanosomes of the *brucei-gambiense* group than are most of the species of animals upon which tsetse feed. In the case of cattle, for example, it is well known that the briefest exposure of the animals in a fly-belt results in their becoming infected; but numbers of

natives have lived for years in contact with game-tsetses without mishap. Apparently the great majority, if not all, of the cyclically-carried wild-fly strains of polymorphic trypanosomes in game-tsetse areas are innocuous to healthy man. Yet there exists, in some of these areas, a certain number of human beings who carry in their blood trypanosomes indistinguishable from the ubiquitous game organism. An attempt has been made elsewhere to discuss the etiology of these human strains (Duke, 1923b). The German view is that the human parasite, the so-called *T. rhodesiense*, is specifically different from *T. brucei*. The ability to infect man is regarded as possessing the importance of a specific character, and therefore, presumably, sufficiently deep-rooted to survive cyclical passage through tsetse. Now there is no evidence, as far as I know, to warrant this assumption. If such a genuine human parasite existed, regularly transmissible from man to man cyclically by *Glossina*, we should expect to find a considerably larger number of cases of human trypanosomiasis than actually occur in the populations of infected *morsitans* areas. We have no evidence of the existence in man in these game-tsetse areas of a mild form of trypanosomiasis analogous to that found in game animals.

There is obviously a hitch somewhere in the progress of the trypanosome as a human parasite in these regions. Its behaviour in man and the curious distribution of the disease are not adequately explained by the theory that the parasite relies mainly, if not entirely, on cyclical transmission for its passage from man to man. On the other hand, several facts point to the intervention of direct transmission. The human host succumbs to a fatal disease, in the course of which the peripheral blood is heavily charged with trypanosomes. The clinical condition is thus favourable to direct transmission, and this is further facilitated by man's gregarious habits and his hairless skin. By direct transference to another human host the trypanosome evades the ordeal of cyclical passage through the tsetse, and the attendant risk of losing its precarious hold on man.

We have seen above that a strain may undergo several passages by direct transmission without impairment of its transmissibility by the cyclical method. Possibly this observation has an important bearing on the problem before us. A series of direct passages from man to man may confer on a strain of trypanosomes, attributes

sufficiently deep-rooted to survive cyclical passage through *Glossina*. In this way more or less stable cyclical human strains might arise in the wake of an epidemic.

Direct transmission offers an explanation of the sporadic distribution of the human disease in certain *morsitans* areas. In the presence of tsetse, a sick man whose blood is swarming with trypanosomes will afford excellent opportunities for the direct transference of his blood, by the fly, to his immediate neighbours ; whereas those of his trypanosomes which succeed in establishing themselves cyclically in the tsetse may, on the completion of their development in the fly, be no longer able to survive in man's blood.

There is a widespread tendency to assume that all human cases derive their infection from cyclically-infected wild flies. Great stress is laid on the fact that so-and-so became infected shortly after visiting a fly-area to hunt ; but the facts that nearly every male in the area is a hunter, and that the patient himself or his friends and relations live in more or less close contact with tsetse, are apt to be overlooked.

In such a community, parties of natives will visit the uninhabited fly-country from time to time to hunt, or to cut poles, or for some other objective, and there is nothing to prevent an early case of trypanosomiasis from participating in these excursions. In the presence of hungry tsetse such a man is a menace to his fellows. One can hardly imagine conditions more ideal for the distribution of the sick man's trypanosomes by the direct method than those obtaining during the later stages of a native hunt in *morsitans* country. I believe that a 'clean' native entering one of the so-called infected *morsitans* areas is much more likely to be infected by the fly if one or more of his companions are already suffering from trypanosomiasis, than if he goes into the fly-country alone or with 'clean' companions. Until we have irrefutable evidence that 'clean' natives, from a 'clean' area where there is no fly and no human trypanosomiasis, can contract the disease by entering a fly-area where there are no people, we should at least maintain an open mind about the relative importance of cyclical and direct transmission in the spread of human trypanosomiasis in game-tsetse areas.

In the present state of our knowledge we may suppose that,

under circumstances which are not yet understood, the game trypanosome, *T. brucei*, can establish itself in man, using him as an adventitious host and causing a fatal disease, a feature of which is a heavy infestation of the peripheral blood. The parasite thus stands an excellent chance of being transmitted mechanically to fresh human hosts, and of maintaining intact those qualities, whatever they may be, which originally determined its establishment in man.

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# EXPERIMENTAL STUDY OF TRYPANOSOMIASIS IN PALESTINE

BY

I. J. KLIGLER

AND

I. WEITZMAN

(*From the Laboratory of the Malaria Research Unit, Department of Health, Haifa*)

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## I. EXPERIMENTAL TRANSMISSION, SYMPTOMS AND PATHOLOGY

There is little information extant regarding the prevalence of trypanosomiasis in Palestine. There are no records of its existence prior to the war, but during the war trypanosomes were reported to have been found in army camels. Goldberg (1917) reports a severe epidemic in 1915, among the camels of the Turkish Army, during which 462 out of 956 camels died. The author classes the trypanosomes as *T. evansi*. Stuart (1923) reports that he found trypanosomes in a number of sick camels belonging to the army of occupation, which he also identified as *T. evansi*. There is, however, no record of this disease in native horses and mules.

Recently Dr. Deuel, a local veterinary surgeon discovered an outbreak of trypanosomes in mules on a farm in Northern Palestine. The nature of the disease was, apparently, not clear, since the veterinarians differed with regard to its character ; some claimed it was Nagana, while others classed it as Dourine ; Surra was, apparently, not suspected. In order to clear up this matter we undertook a study of the disease.

History of the outbreak. In September, 1922, an English mare and a native mule became ill with what appeared to be haemoglobinuria, but laboratory examinations established the presence of trypanosomes in the blood. In January, 1923, three more mules became ill and shortly after another one, making a total of 6 out of 15 animals. The symptoms were : emaciation, swelling of the breasts, intermittent fever, and occasionally haemoglobinuria. The diagnosis of trypanosomiasis was made and confirmed by blood films examined in the Hadassa Laboratory at Saffed, and by Dr. Stuart in the Central Government Laboratory. Three of the mules were native animals and two were imported from Syria ; the mare was bought in Saffed, but its past history was not known. All the animals, but the mare, had been on the farm two or three years prior to the outbreak of the illness ; the mare was the only animal acquired about four months before the discovery of the illness.

Transmission of the Infection. Thanks to the assistance of the Chief Veterinarian of the Department of Agriculture, we obtained two of the sick mules, one male and one female, for our studies.

The animals were brought to the laboratory yard and kept there for observation.

At the time of their arrival neither of the animals had trypanosomes in the blood. Nevertheless, blood was inoculated into two dogs and successful transmission obtained after an incubation period of seven days.

Subsequent to the first transmission experiment and prior to the appearance of parasites in the blood of the infected animals, we were advised by a local veterinarian (Dr. Freund) to try the effect of arecolin on the mobilization of trypanosomes in the peripheral circulation. The results were quite interesting. A dose of 0·05 gm. injected subcutaneously caused, almost immediately, marked salivation, defecation and urination. Forty-five minutes after the injection the blood was still negative, but after one hour trypanosomes were present in the peripheral blood. The drug had the identical effect in both mules.

After the appearance of trypanosomes in the blood, a second series of transmission experiments was made from both mules into dogs, rabbits and guinea-pigs. The blood was taken with a syringe from the jugular vein, a portion of it was mixed with 1·5 per cent. citrate solution, and varying amounts of the whole and citrated blood were inoculated intraperitoneally into animals as indicated in the table below. All of the animals showed trypanosomes in the blood after an incubation of six to eight days, with the exception of one guinea-pig in which the incubation period was eleven days.

Table I

Transmission of Trypanosomes from Infected Mules to Lower Animals

| | Mule 1 (black, male) | | Incubation | Mule 2 (white, female) | | Incubation |
|---|---|---|---|---|---|---|
| | No. | Dose | | No. | Dose | |
| Dogs ... ... | 1 | 5 c.c. whole blood | 6 days | 1 | 5 c.c. citrated blood | 7 days |
| | | | | 1 | 10 c.c. ,, ,, | 6 ,, |
| Rabbits ... | 1 | 3 c.c. ,, ,, | 7 ,, | 1 | 3 c.c. ,, ,, | 7 ,, |
| Guinea-pigs ... | 2 | 2 c.c. ,, ,, | 7 & 8 ,, | 2 | 2 c.c. ,, ,, | 8 & 11 ,, |

the guinea-pig, the course of the disease was more acute and toxic in character ; often the guinea-pig died from the infection, without showing any external signs whatever, other than progressive loss of weight.

NATURE OF THE DISEASE. The evidence presented by our observations on the naturally and experimentally infected animals leads us to believe that we are dealing with Surra, and that the trypanosomes responsible for the condition belong to the *T. evansi* group. In morphology the organisms belong to the monomorphic group. They are slender, actively motile trypanosomes, 18 to 22$\mu$ long and 1·5 to 2·0$\mu$ wide. The posterior extremity is slightly pointed ; the undulating membrane is well-defined ; the flagellum

TABLE II

Blood Picture in Experimental Trypanosomiasis in Guinea-Pigs
(Inoculated October 22, 1923)

| | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|
| W.B.C. ... ... ... | 9,600 | 10,000 | 8,400 | 6,000 | 5,200 | 4,800 | 5,600 | 5,600 |
| Polynuclears (%) ... | 69 | 67 | 50 | 42 | 37 | 32 | 37 | 40 |
| Lymphocytes (%) ... | 28 | 30 | 46 | 55 | 59 | 63 | 59 | 55 |
| Large mononuclears and transitionals (%) ... | 3 | 3 | 4 | 3 | 4 | 5 | 4 | 5 |
| Blood picture ... ... | normal | normal | anaemic 'crescents' | polychromatosis, 'crescents,' normoblasts. | | | | |
| Trypanosomes ... ... | — | — | — | — | + | + | + | + |

is free and fairly long—about one-third or less the length of the parasite. The nucleus is large, oval and central ; the blepharoblast is round and situated about one-quarter to one-third of the distance between the posterior end and the nucleus. The protoplasm is uniform or slightly granular.

The incubation period in experimental animals of five to seven days, the pathogenicity for the dog, rabbit and guinea-pig, the inability to transmit the infection by coitus and the picture of the spontaneous and experimental disease correspond fairly closely with that observed by other investigators in Surra.

CULTIVATION OF THE TRYPANOSOMES. Cultivation experiments

thus far have failed completely. A large variety of media have been tried, including the classic N.N.N. medium, the medium used by one of us (Kligler, 1924) in the cultivation of Leishmania, Noguchi's media and various modifications; but the results have thus far been uniformly negative. The only result obtained was survival of trypanosomes for three days in normal rabbit serum incubated at 22° to 26° C.

## II. EFFECT OF 'BAYER 205' IN EXPERIMENTAL TRYPANOSOMIASIS

Curative Power of the Drug. In our experiments we used dogs, rabbits, and guinea-pigs. Although mice are often preferred in chemo-therapeutic experiments on account of the ease with which parasites are found in the blood, the disease in the rabbit and the guinea-pig resembles more closely that in the mule than does the infection in mice; the former are, therefore, more satisfactory for therapeutic tests. We also found that, using the thick drop method, it was not at all difficult to find the trypanosomes in the blood of rabbits and guinea pigs even if present only in small numbers. The blood picture in these animals is another valuable aid in following the course of the infection; in the guinea-pig, at least, we have found it almost as diagnostic as the presence of parasites. Leucopaenia and anaemic elements (Crescents, Cabot's rings, etc.) are constant throughout infection, but disappear when the animal is cured. It is possible, therefore, by the combined method of thick drop examination and a study of cellular elements of the blood to follow without difficulty the course of the infection and the effect of the drug.

In testing the therapeutic value of the drug, we tried to determine the minimum single dose which will uniformly sterilise infected animals. The doses varied from 0·2 gm. to 0·04 gm. per kilo of body weight. The injections were made intraperitoneally from 5 per cent. or 10 per cent. solutions of the drug.

In the course of these experiments we treated a large series of guinea-pigs and rabbits in various stages of the illness, with varying amounts of the drug. Doses of 0·1 gm. per kilo or over sterilized all rabbits and guinea-pigs without exception. A dose of 0·05 gm. per kilo of body weight did not yield 100 per cent. of cures. Of fifteen

guinea-pigs treated with this dose two relapsed, one three days and the other fourteen days after treatment. Similarly one of five rabbits treated with this dose of the drug relapsed after eleven days. In all cases of relapses the condition of the animal rather than the duration of the illness seemed to be the important element. The two guinea-pigs had received the infection twenty-four days and the rabbit twenty days before treatment ; other animals treated at the same stage of the infection recovered completely.

It is evident from these experiments that a single dose of 'Bayer 205' is capable of sterilizing the blood of dogs, rabbits and guinea-pigs infected with a virulent strain of trypanosomes (*T. evansi*) with the apparent production of a permanent cure. The drug was administered at various stages of the infection, always with the same results.

Animals in the later stages of the infection, showing definite physical signs of the disease at the time of treatment, recovered completely and these signs cleared up within a few days after the drug was administered. Two of the guinea-pigs had advanced skin lesions at the time of treatment and within a few days the ulceration ceased and the wounds healed progressively. The smaller doses of the drug (0·05 gm. per kilo) were more satisfactory, despite the fact that a few of the animals relapsed, because of the practical absence of any noticeable toxic effect. The large doses gave more uniform results, but, as will be noted below, they were not far removed from the toxic dose of the drug, and often they actually produced toxic symptoms.

Toxic Effect of 'Bayer 205.'—In the course of our experiments, we have had occasion to note that the drug is highly toxic for rabbits and guinea-pigs. Several of our animals which were treated with the larger doses (0·1 to 0·2 gm. per kilo) died within a short period after the administration of the drug without any apparent clinical cause. Two rabbits died within six and twelve days and three guinea-pigs within two, six and seven days, respectively, after the injection of the drug. We, therefore, injected various doses of the drug into a series of animals in order to determine the lethal dose of the drug and the pathological changes produced in the body. A dose of 0·4 gm. per kilo of body weight injected intraperitoneally killed a medium size guinea-pig (460 gms.) in four days. Smaller

doses are not uniformly lethal ; a guinea-pig which received 0·25 gm. of the drug per kilo died seventeen days after the injection with typical pathological changes, while at the same time another guinea-pig receiving 0·3 gm. per kilo remained alive and well. These tests show that, for the guinea-pig at least, the therapeutic dose is approximately one-fifth to one-eighth of the lethal dose. But even smaller doses (0·1 to 0·2 gm. per kilo), which may have little effect on normal guinea-pigs or rabbits, may be decidedly toxic when injected into animals infected with trypanosomes.

Pathological Changes produced by the Drug.—In the infected animals it was difficult to differentiate the changes due to the infection from those caused by the drug. The infected animals which died after treatment showed a greater or lesser degree of degeneration of the kidney tubules, but this type of lesion was also characteristic of animals which died from a trypanosome infection. The changes found in the control guinea-pigs may be accepted as characteristic of the lesions produced by the drug. The principal lesion was extensive degeneration of the cells of the tubules, chiefly in the cortical region. The blood vessels were also congested. Another striking feature was a deposition of a brown pigment in the spleen. The liver was congested and there were, here and there, small areas of necrosis, but the changes were not as striking as those in the kidneys. The lesions found in the kidney taken with the reported presence of albuminuria in patients treated with ' Bayer 205 ' suggest a selected toxic affinity of the drug for the kidney tubules. Recently Duncan and Manson-Bahr (1924) reported almost identical changes in mice injected with lethal doses of the drug.

Prophylactic Effect of ' Bayer 205.'—Mayer and Zeiss (1921) emphasize the fact that ' Bayer 205 ' remains a long time in the animal body and consequently serves as a prophylactic against infection. Brumpt (1924) has made similar observations. These authors worked with mice. Kleine (1924) working with larger animals, failed to get protection against infection. We have tested the prophylactic effect of various doses of the drug in rabbits and guinea-pigs. The following are typical protocols :—

Rabbit Y. Weight 1390 gms. received on 1.7.23, intraperitoneally 0·10 gm. ' Bayer 205 ' per kilo of body weight. On 1.8.24, one month later, the rabbit received an infective dose of trypanosome blood. The blood of the rabbit remained negative. One month later this rabbit received another dose of infected blood,

and after an incubation period of 10 days, trypanosomes appeared in the circulation.

Rabbit 17. Weight 1680 gms., 0·05 gm. per kilo 'Bayer 205' given intraperitoneally, 16.12.23. This was followed after 25 days by an injection of trypanosomes. The result was negative. Sixty-three days later another infective dose given; after 15 and 18 days trypanosomes were found in the blood; since then the blood remained negative, but the animal developed all the clinical signs of chronic trypanosomiasis.

Rabbit 15. In this case the infective dose was given three days before the injection of 0·05 gm. 'Bayer 205' per kilo. Twelve days after the infection trypanosomes appeared in the circulation for one day. After that the blood remained negative, although clinical symptoms developed. Five weeks after the first appearance of trypanosomes the parasites were again found in the blood, and they persisted intermittently until the death of the rabbit.

Guinea-pig X. Weight 440 gms., received 0·2 gm. 'Bayer 205' per kilo, intraperitoneally and one month later an infective dose of trypanosomes. After a somewhat prolonged incubation period of 15 days, trypanosomes appeared in the circulation.

Guinea-pig 34. Weight 440 gms., 0·3 gm. 'Bayer 205' per kilo injected intraperitoneally on 16.11.23. After 25 days an infective dose of trypanosomes inoculated intraperitoneally. Trypanosomes found once after 36 days and thereafter the animal was normal. After 88 days the animal was reinfected a second time, but no signs of infection developed during the month following the infection. The animal died from a bile injection.

These observations indicate that the drug affords a considerable protection, and that the degree and duration of the protection varies with the amount of drug administered and the individual animal. It is noteworthy that the drug given shortly after the infection, that is, during the incubation period, did not prevent the development of the disease.

Trypanocidal Action of 'Bayer 205' in vitro.—Tests made *in vitro* to determine the trypanocidal action of this drug gave totally different results from those obtained *in vivo*. In the test tube a concentration of 1 : 100 of the drug failed to immobilize this strain of trypanosome in five to eight hours; while in the animal body 0·05 gm. per kilo (a dilution of 1 : 2000 or less) sterilized the blood of infected animals in fifteen hours.

Several modified procedures were employed in these experiments. In one method blood was drawn from an infected animal into a test tube containing a few glass beads. The blood was defibrinated and divided into small Wasserman tubes : 0·9 c.c. into the first tube and 0·5 c.c. into four or five others. 0·1 c.c. of a 10 per cent. solution of 'Bayer 205' was added to the first tube and further dilutions made by adding 0·5 c.c. from tube 1 to tube 2, etc. This gave a series

of dilution of 100, 200, 400, etc. The tubes were kept at 25° C. and drops examined under the dark field microscope at various intervals. This was the method of choice, because the trypanosomes remained in a favourable medium and the conditions approximated those in the animal body.

Another method consisted in the use of infected citrated plasma from which the red cells had been removed by centrifugalization for five to six minutes at 500 revolutions per second. By slow speed centrifugation the red cells are removed, while a sufficiently large number of actively motile trypanosomes remain in the supernatent plasma for the purpose of the experiments. The rest of the procedure was the same as with the defibrinated blood.

A third method consisted in the use of normal rabbit or guinea-pig serum, containing various dilutions of the drug to which small amounts of infected blood or serum were added.

Whichever method was employed the results were the same. During the first four to eight hours no effect was noted on the motility of the trypanosomes in dilutions of 1 : 100 or more. After twenty-four hours, active trypanosomes were still encountered in the 1 : 100 dilution, but the number was considerably less than in the control tubes. After forty-eight hours no motile trypanosomes were found in the dilution of 100 and 200, but the higher dilutions still contained actively motile trypanosomes.

Similar observations were made with bacteria. Contaminating air bacteria as well as *B. coli* multiplied actively in tubes containing 1 : 100 dilution of the drug.

It is apparent from these results that under the conditions of the experiments the drug is only slightly trypanocidal. This fact is of no particular importance in so far as the therapeutic value of the drug is concerned. It is of interest, however, in relation to the mechanism of the action of the drug. It is clear that either 'Bayer 205' undergoes some change in the body which renders it an effective trypanocide, or that it acts indirectly on the trypanosomes by inducing certain changes in the resistance of the host. In this connection it is noteworthy that the injection of 'Bayer 205' is followed by a marked increase in the total leucocyte count as well as in the large mononuclears; in some animals this increase is very large and persistent.

## III. MECHANISM OF RESISTANCE TO TRYPANOSOME INFECTION

Resistance of Cured Animals to Reinfection.—It is well-known that rats which recover spontaneously from an infection with *T. lewisi* are immune to a reinfection with that organism. It remained to determine whether recovery from an infection with a pathogenic trypanosome will also confer immunity on these animals. We realised that the analogy was not absolute, since our animals did not overcome the infection naturally as in the case of the rats. Nevertheless, we investigated this point by attempting to reinfect guinea-pigs and rabbits cured with 'Bayer 205.'

At various intervals after treatment with 'Bayer 205' the cured animals were injected with infective blood. These reinfections were repeated at various intervals under different conditions. All of the animals, without exception, showed a marked degree of resistance to reinfection, although the duration of this resistance varied. The resistance of guinea-pigs was apparently more persistent than that of rabbits. Several of the animals died after the second or third attempt at reinfection, but none of them showed any clinical signs of infection at the time. Reinfections were obtained in three rabbits out of the entire series and in these cases the resistance was apparently broken down artificially.

Table III (p. 449) gives a summary of the reinfection experiments, showing the duration and degree of the resistance in the different animals.

It is apparent from these results that infected guinea-pigs and rabbits cured with 'Bayer 205' develop a resistance to reinfection of long duration. In rabbits this resistance lasts three to four months, while in guinea-pigs it persists for longer periods.

Nature of Resistance.—The experiments recorded in Section 2 indicate that 'Bayer 205' injected into normal animals protects guinea-pigs and rabbits for a greater or lesser period, depending on the amount of the drug injected. This protection was not, however, as lasting as that observed in infected animals cured with 'Bayer 205.' In cured animals long-standing resistance develops even when treated with so small a dose as 0·05 gm. per kilo of body weight. Kleine and Fischer (1922), working with monkeys, also found that

cured animals developed a more persistent resistance to reinfection than those given a prophylactic dose of the drug.

In view of the bearing that this phenomenon may have on the general problem of immunity or resistance to protozoan infections, we attempted to examine more closely the mechanism of this resistance to reinfection in our cured animals.

Table III

| Animals | | Date of infection | Date of treatment | Dose per kilo | Reinfections: Days after treatment | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | |
| Rabbit | 7 | 10.6.23 | 2.7.23 | 0.1 | 29 | 65 | 82 | 132* | 20 days after last reinfection parasites appeared in the blood. |
| ,, | 8 | 25.6.23 | 2.7.23 | 0.1 | 29 | 65 | — | — | 22.10; 110 days after treatment animal died, when blood was drawn from heart. Infection negative. |
| ,, | 14 | 26.11.23 | 16.12.23 | 0.05 | 35 | 99 | 160* | — | After third reinfection, infection developed and followed a normal course. |
| Guinea-pig | 1 | 8.4.23 | 27.4.23 | 0.2 | 44 | 76 | 95 | 131, 178 | 10.11; 197 days after treatment animal well. |
| ,, | 8 | 21.6.23 | 2.7.23 | 0.2 | 29 | 65 | — | — | 18.9; 78 days after treatment animal died of pneumonia. |
| ,, | 9 | 25.6.23 | 15.7.23 | 0.15 | 16 | 52 | — | — | 5.10; 82 days after treatment animal died while being bled. |
| ,, | 35 | 10.12.23 | 27.1.24 | 0.05 | 56 | 116 | — | — | Died from extraneous cause 146 days after treatment; no infection. |
| ,, | 38 | 10.12.23 | 27.1.24 | 0.05 | 56 | 116 | — | — | Observed 170 days, still alive and well. |
| ,, | 104 | 17.1.24 | 17.2.24 | 0.10 | 36 | 120 | — | — | No infection, died after observation 147 days, from sunstroke. |
| ,, | 106 | 28.1.24 | 18.4.24 | 0.06 | 39 | — | — | — | Alive and well, no signs of infection. |

* Parasites appeared only after an injection of oil.

*Humoral antibodies.*—It is well known that the serum of rats which have recovered spontaneously from the infection with *T. lewisi* has the power to agglomerate trypanosomes. A similar condition has not been observed in animals infected with pathogenic trypanosomes. Levaditi and McIntosh (1910) have reported the

presence of a trypanocidal antibody in trypanosome infected animals following a pyrexial period. It appeared, therefore, of interest first of all to note whether there were any of the humoral antibodies in our cured and resistant animals. For this purpose a series of experiments were performed of which the following are examples. Each experiment was repeated at least two or three times, but only type experiments are given below :—

Experiment 1. Sera were collected from treated animals at intervals of 3 to 8 weeks after recovery. These sera were diluted with saline in various proportions up to 1 : 10 and suspensions of live trypanosomes added. The suspensions of trypanosomes were prepared as follows :—5 c.c. of blood from a heavily infected animal were taken from the heart and mixed with an equal volume of 1 per cent. citrated Ringer solution. The red cells were then sedimented by slow centrifugation—about 600 revolutions for 5 to 6 minutes. The supernatant fluid contained a uniform suspension of trypanosomes. Definite quantities of the suspensions were added to the sera and the tubes placed at 20° to 25° C. Observations for motility and agglutination were made with the dark field microscope, at various intervals, from 1 to 48 hours. Controls were made with sera from normal animals and from infected animals taken shortly after pyrexial crisis.

In none of the experiments was there any indication of injury to, or even retardation of, the motility during the first six hours. In all of the higher concentrations of serum up to 1.5, actively motile trypanosomes were seen even after an incubation of 48 hours. In general the sera from cured resistant animals reacted in the same manner as did normal sera.

Experiment 2. This experiment was an attempt to apply the Pfeifer reaction to the trypanosomes. Sera from resistant animals were mixed with suspensions of trypanosomes made in the manner described above in the ratio of 5 to 10 parts of serum, respectively, to one part of suspension. The mixtures were injected intraperitoneally into guinea-pigs and, at intervals, material was withdrawn for dark field observation.

There were two variations to this experiment.

Experiment 2a. Two guinea-pigs were each inoculated with 1·0 c.c. serum from cured guinea-pig No. 1 and at the same time 0·1 c.c. of infected blood was injected. A control animal was treated in the same manner with normal serum. Material was withdrawn from the peritoneal cavity at intervals of half, 1, 2, and 18 hours for dark field examination. The trypanosomes remained actively motile, although after 18 hours the stained preparations of the trypanosomes from all the animals showed vacuolations. After an incubation period of 10 to 11 days trypanosomes appeared in the circulation.

Experiment 2b. Sera from three cured resistant animals were pooled and 5 c.c. quantities mixed in varying ratios with trypanosome suspensions. The suspensions were incubated for one hour at 25° C. and then injected into guinea-pigs of approximately the same weight. At intervals similar to those in Experiment 2a, specimens were withdrawn with a capillary pipette and examined under the dark field microscope.

There was no apparent effect on the motility during the twenty-four hours after the injection. Seven to eight days after the injection trypanosomes appeared in the circulation of all of the animals used for the experiment.

This series of experiments indicated that the blood of resistant animals did not contain any trypanocidal antibody which could be demonstrated either *in vitro* or *in vivo*.

*Cellular factors in resistance to reinfection.*—Immunity is usually considered a two-fold phenomenon—cellular and humoral. In the absence of any demonstrable humoral antibodies we directed our attention to the cellular changes occurring during the infection and subsequent to treatment. It was noted that marked and striking cellular changes occur. The following table (Table IV) gives a summary of white cell counts of a series of animals before and during the infections and before and after treatment. It will be noted that during the infection there is a moderate but uniform leucopaenia with a preponderance of lymphocytes, and that immediately after treatment there is a sharp increase in the total leucocyte count and in the large mononuclears, and a tendency to a reversal of the ratio to normal. This increase is usually greater than normal and the condition persists in all the cured animals for a considerable period.

The bearing of Relapses to Resistance to Reinfection.—It was noted above that the course of the disease in the mule as well as in the experimental animals is intermittent in character ; pyrexia accompanied by the appearance of trypanosomes in the circulation is followed by apyrexial periods of greater or lesser duration. In the rabbit these apyrexial intervals are much greater than in the guinea-pig and occasionally a rabbit may develop a severe type of chronic infection with emaciation, loss of hair, conjunctivitis, keratitis, etc., and yet parasites may be rarely encountered in the peripheral circulation.

The explanation of the relapsing nature of the infection is obscure, but it is undoubtedly bound up in some way with the resistance of the animal host to the invading virus. It appears likely that if not the same, at least similar factors play a rôle in the two phenomena : the resistance to blood invasion and resistance to reinfection ; and that an elucidation of the factors determining the appearance of relapses might throw light on the nature of the immunity.

TABLE IV

Blood picture in Experimental Trypanosomiasis before and after Treatment*

| No. of animal | Average Counts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | During incubation | | | | During infection | | | |
| | Total | Poly-morphs | Lympho-cytes | Mono-nuclears | Total | Poly-morphs | Lympho-cytes | Mono-nuclears |
| Guinea-pig 104 | 11,300 | 65 | 34 | 4 | 8,200 | 46 | 52 | 3 |
| ,, 107 | 8,200 | 60 | 35 | 3 | 7,000 | 40 | 58 | 2 |
| ,, 112 | 11,100 | 68 | 31 | 3 | 7,000 | 52 | 42 | 5 |
| Guinea-pig 32 | 8,000 | 52 | 45 | 3 | 8,000 | 37 | 58 | 5 |
| ,, 33 | 10,000 | 60 | 35 | 2 | 10,000 | 48 | 49 | 2 |
| ,, 35 | 9,400 | 58 | 40 | 2 | 9,200 | 41 | 56 | 3 |
| ,, 38 | 8,400 | 63 | 35 | 2 | ... | ... | ... | ... |
| Rabbit 8 | 8,000 | 55 | 43 | 2 | 6,500 | 29 | 68 | 3 |
| ,, 11 | 8,000 | 60 | 38 | 2 | 7,500 | 54 | 44 | 2 |
| ,, 18 | 8,000 | 58 | 40 | 2 | 7,000 | 38 | 60 | 2 |
| ,, 14 | 9,000 | 65 | 33 | 1 | 7,400 | 37 | 61 | 2 |

| No. of animal | Average Counts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | After treatment | | | | Immediately after 'Bayer 205' | | | |
| | Total | Poly-morphs | Lympho-cytes | Mono-nuclears | Total | Poly-morphs | Lympho-cytes | Mono-nuclears |
| Guinea-pig 104 | 10,000 | 58 | 38 | 4 | 10,000 | 78 | 16 | 6 |
| ,, 107 | 7,800 | 60 | 39 | 4 | 8,000 | 68 | 24 | 7 |
| ,, 112 | 9,700 | 61 | 36 | 3 | 9,200 | 45 | 40 | 15 |
| Guinea-pig 32 | 12,000 | 51 | 45 | 4 | 10,900 | 38 | 53 | 9 |
| ,, 33 | 10,200 | 63 | 32 | 5 | 8,600 | 37 | 54 | 9 |
| ,, 35 | ... | ... | ... | ... | 9,200 | 38 | 57 | 5 |
| ,, 38 | 9,800 | 53 | 43 | 4 | 12,200 | 48 | 47 | 5 |
| Rabbit 8 | 11,000 | 56 | 42 | 2 | 11,000 | 56 | 40 | 3 |
| ,, 11 | 11,500 | 51 | 46 | 3 | 9,200 | 58 | 35 | 7 |
| ,, 18 | 9,700 | 47 | 51 | 2 | 10,200 | 60 | 34 | 6 |
| ,, 14 | 10,000 | 45 | 52 | 3 | 9,900 | 60 | 36 | 4 |

* The averages are obtained from approximately ten counts made during the infection or after the treatment, and the entire incubation period. It should be noted that during the pyrexial periods there is a marked lymphocytosis which gradually changes to a normal differential during the apyrexial period. In treated animals the maximum total counts are obtained two or three days after the injection of the drug. During the first few days the lymphocytes and large mononuclears predominate, but gradually the cell distribution approaches normal, although the total count may be considerably above the normal average.

Assuming that this interplay of host resistance to virus is associated with the cellular mechanism, we attempted to induce relapses artificially by agents which are known to affect the white cell equilibrium. The work of Bergel (1921) and the results obtained by Nakahara (1922) in his studies on cancer, indicated that oil, particularly olive oil, may produce marked changes in the white cell formula and in the resistance of the host to an invasion of foreign bodies. We consequently used a variety of oils with striking results.

Artificial Production of Relapses.—Relapses were produced at will by the injection of oils, such as cod liver or olive oil, particularly the latter. Ordinary commercial olive oil was employed. The oil was sterilized in the autoclave and injected intraperitoneally. As the protocols indicate, the effect of the oil has been tried on a fairly large series of animals under various conditions and the results have been unfailing in their constancy.

The following are brief extracts of the protocols :

*A. Experiments with guinea-pigs.**

1. *Effect of small doses of olive oil :*

Guinea-pig 13. Infected 1 July ; infection of long duration ; parasites intermittently present in the circulation. On 11 November parasites negative ; 0·25 c.c. sterile olive oil given intraperitoneally. 12 November large number of trypanosomes in the blood ; persistent until 16 November, then negative until 30 November ; 0·25 c.c. oil again given. On 3 December trypanosomes positive and increased steadily in number assuming appearance of continuous infection, until 16 December when treated.

Guinea-pig 26. Infected 22 October ; 26 October blood positive ; remittent infection. On 11 November rare parasites present in the circulation ; given 0·25 c.c. of olive oil i.p. ; on 12 November trypanosomes positive and number increased until 14 November, when the trypanosomes suddenly disappeared, and the blood continued negative.

Guinea-pig 28. Infected 22 October ; parasites infrequently present in small numbers. On 11 November blood negative ; 0·25 c.c. olive oil given intraperitoneally ; on 12 November trypanosomes appeared in the circulation, persisted until 15 November when treated.

2. *Effect of large doses of oil.*

Guinea-pig 12. Infected 19 July ; trypanosomes appeared in the blood on 29 July ; present at irregular intervals for varying lengths of time ; infection unusually long duration. On 11 November trypanosomes were negative ; 4 c.c. olive oil injected i.p. ; 12 November trypanosomes found in the blood. Infection

* The weights ranged from 450 to 500 gms.

positive to 15 November, then negative until 25 November and intermittent. On 30 November another injection of 4 c.c. oil given, 2 December after two days, there was a large increase in the number of parasites and they persisted to the end on 18 December.

Guinea-pig 24. Infected 22 October; 26 October blood positive; intermittent infection. 11 November few parasites in the circulation; injected 4 c.c. olive oil; 12 November large increase in numbers; trypanosomes persisted for seven days and then disappeared. After the blood was negative 11 days, another injection of 4 c.c. given; two days later parasites reappeared in the blood and persisted until 16 December, when treated.

Guinea-pig 27. Infected 22 October, remittent infection. On 11 November blood negative; 4·0 c.c. olive oil injected intraperitoneally. 12 November trypanosomes present in the circulation; persisted until 15 November when animal treated.

Guinea-pig 31. Infected 10 December, trypanosomes in blood 16 December; then negative until 15 January. That day 4 c.c. olive oil injected intraperitoneally; 16 January trypanosomes appeared in the blood and continued present until 17 February when treated.

Guinea-pig 36. Infected 10 December; 18 December blood positive. Examined at intervals until 15 January and no parasites found. On 15 January 4 c.c. olive oil given i.p. On 16 January trypanosomes appeared in the circulation and continued positive until 3 February, when enormous numbers were found in the blood and animal treated.

Guinea-pig 38. Infected 10 December. Blood continually negative. On 15 January 4 c.c. olive oil given i.p.; next day trypanosomes appeared and persisted with two days intermission until 27 January when treated.

### *B. Experiments with Rabbits.*

Rabbit Y. Weight 1390 gms. Infected 5 September. Blood positive 17 September. Parasites rare in circulation; present only five times in two months. On 11 November 5 c.c. olive oil given i.p. On 13 November parasites appeared in circulation, increased in numbers until 18 November; then negative for five days; then increasingly positive until 27 November when the animal died with a heavy blood infection—a most unusual occurrence in rabbits.

Rabbit 4. Weight 1650 gms. Infected 5 November, course of infection irregular; relapses at irregular intervals. On 21 December 10 c.c. olive oil given i.p.; 22 December, trypanosomes present; infection continuous until the animal died.

Rabbit 19. Weight 1250 gms. Infected 10 November, acute conjunctivitis and loss of hair, but trypanosomes constantly negative. On 25 February, 4 c.c. olive oil given i.p.; on 27 February blood was positive and continued so until 3 March, when treated.

The preceding protocols indicate that olive oil injected intraperitoneally into infected guinea-pigs and rabbits was followed usually within twenty-four to forty-eight hours, by an appearance of trypanosomes in the peripheral circulation. The immediate results are not affected by the amount of oil injected; but there seems to be

some relationship between the amount of oil and the persistence, or continued multiplication, of the trypanosomes in the blood. The smaller doses (0·25 c.c.) were followed by a relapse, the persistence of parasites for three or four days, followed by their disappearance for a greater or lesser interval. The larger doses of oil were also followed by the appearance of trypanosomes in the circulation within twenty-four to forty-eight hours after the injection, but the persistence was apparently longer than was the case with the small doses, and in many of the animals the resistance seemed to have been broken down completely. Whenever parasites were present in the body the injection of olive oil served to cause a flare-up and an invasion of the circulation. Rabbits reacted in the same way as guinea-pigs.

Suppression of Resistance in Cured Animals.—Proceeding on the hypothesis that there is a relationship between the resistance to blood invasion and that to reinfection, we attempted to determine whether it is possible to break down the resistance in the same manner as it was possible to produce relapses. Into animals cured from an infection were injected varying doses of olive oil in a manner similar to that used to produce relapses. The results were not uniformly successful, but in a few cases we actually succeeded in breaking down the resistance and calling out an infection which had apparently remained latent. The observations are given in the following protocols :

Rabbit 11. Weight 1750 gms. Injected 26.11.23 ; on 29.11.3, 22 c.c. of bile injected intraperitoneally. Trypanosomes appeared 1.12.23, the rabbit was treated with 0·05 gm. ' Bayer 205 ' per kilo. The blood was rendered negative, and the blood picture changed. On 15 January 4 c.c. oil was given intraperitoneally. On 16 January trypanosomes were found.

Note. The drug had not destroyed the trypanosomes, but rendered the infection latent. A dose of oil caused a flare up of the infection.

Rabbit 7. After three superinfections with negative results (see Table III) this rabbit was given (on 11.11.23) 6 c.c. oil intraperitoneally together with an infective dose of trypanosomes. No trypanosomes appeared in the circulation. On 30.11.23 a similar dose of oil was given intraperitoneally, and on 1.12.23 trypanosomes were found in the blood. From that day trypanosomes appeared intermittently until 23.12.23, when the animal died from the infection.

Note. In this case it appeared that the oil broke down the resistance and called out the infection which was latent for three weeks.

Rabbit 14. Weight 1690. Infected 26.11.23, treated (0·05 gm. per kilo) 16.12.23. Re-infected 20.1.24, results negative. On 25.3.24, 5 c.c. oil together with 0·1 c.c. infected blood given intraperitoneally. No parasites found in the blood. On 14.4.24 another dose of oil given and on 17.4.24 trypanosomes appeared

in the circulation. They then disappeared until 28.5.24, and from then on continued to appear intermittently until the end.

These results could not be duplicated in guinea-pigs treated with ' Bayer 205.' The reason is not apparent, but it would seem that oil is only effective in mobilizing the trypanosomes when the infection is present in a latent state in the body. In any event, the positive results obtained in rabbits increase the presumption that the resistance to reinfection is a temporary phenomenon bound up in some way with the cellular elements and differing from the type of immunity observed in bacterial infection.

## IV. DISCUSSION

An outbreak of trypanosome infection in mules in Northern Palestine afforded an opportunity for an experimental study of trypanosomiasis in lower animals, particularly rabbits and guinea-pigs.

The morphology of the parasite, the clinical picture of the disease, and the pathological lesions produced, indicate that the strain belonged to the *T. evansi* group.

In the course of these studies the therapeutic effect of ' Bayer 205 ' and the mode of action of this drug were investigated. The drug proved very effective in clearing up the infection in dogs, rabbits and guinea-pigs ; a single dose of 0·05 gm. per kilo of body weight was sufficient to sterilize the blood in all instances and apparently affected permanent cure in 80 per cent. of the rabbits and guinea-pigs so treated.

The mechanism of the action of the drug is of interest. *In vitro* it has very little trypanocidal power ; even a concentration of 1 : 100 requires twenty-four to forty-eight hours to immobilize the parasites under the condition of the test. *In vivo*, on the other hand, so small a dose as 0·05 gm. per kilo suffices to sterilize the blood of a heavily-infected animal in about sixteen hours. It would seem that the drug is either modified in the animal body and rendered more effectively trypanocidal, or that it acts indirectly on the trypanosomes by increasing the resistance of the host. The fact that a small amount of the drug injected into normal animals affords protection against infection for considerable periods, is further presumptive

evidence that the drug either enters into combination with the tissues and is retained in the body for a long time, or that it produces profound changes in the body leading to increased resistance to infection. Which of these alternatives occur it is difficult to say; it is most likely that both events actually take place.

Experimental evidence is advanced in favour of the view that a real immunity or resistance to infection is set up in the body of animals cured from an infection. Even in normal healthy animals there is a partial resistance to an infection with this strain, as indicated by the relapsing nature of the infection. In the course of the infection, the resistance offered to the invasion of the blood stream is repeatedly broken down (probably by substances liberated by the parasites which continue their activity in the tissues), and the trypanosomes penetrate into the circulation and go through a period of active development. The destruction of the trypanosomes in the circulation leads to a partial immunization resulting in an inhibition of growth and a disappearance of the parasites from the circulation.

This immunity or resistance seems to be associated with the formed elements of the blood. Repeated search failed to reveal any of the usual humoral antibodies (agglutinins, lysins). On the other hand, profound changes occur in the white blood cell formula during the infection, and after the treatment. Moreover, it is possible to break down the resistance artificially by the injection of olive oil, a substance shown by various authors to modify the white cell ratio. This observation, namely, the calling forth of relapses by the injection of olive oil, is analogous to the breaking down of the resistance of mice to transplanted cancer by the injection of large doses of olive oil reported by Nakahara (1923). Since, in the latter instance, the resistance has been shown to be due to a proliferation of cells in the lymphoid tissues, and the suppression to a decrease in the lymphoid cells, there is reason to assume that the same or similar factors are involved in the resistance to trypanosome invasions. In this connection, our observation that the injection of 'Bayer 205' causes a marked increase in the white cell count, particularly the large mononuclears, is significant. The defence of the body against trypanosome infections, and perhaps also to other protozoan infections such as malaria, seems, therefore, to lie in the cellular response rather than in the humoral antibodies.

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# NOTES ON SOME TETRARHYNCHID PARASITES FROM CEYLON MARINE FISHES

BY

T. SOUTHWELL

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The material on which this paper is based was collected by the author from various species of Elasmobranch fishes trawled on the Ceylon Pearl Banks during the years 1906 to 1911. A large number of larval forms were also obtained from bony fishes, but, owing to the fact that the proboscides were only rarely extruded, it has not been possible to identify them except in one or two cases. Unfortunately the major part of the writer's collection has been lost and no other material is obtainable; several species obtained by Herdman and Hornell from the same region have been described by Shipley and Hornell in Herdman's Ceylon Pearl Oyster Reports. As far as the writer is aware the only Trypanorhynchids available are in the collections of Professor Pintner of Vienna and Professor Linton in America.

A very large number of Tetrarhynchids have been described from time to time, often from larval forms; the descriptions are, however, so imperfect that it is impossible to identify the majority of these worms again from the meagre details given. Very few of the type species at present exist, with the result that there is great confusion. Until large quantities of material have been collected it will not be possible to revise this Order satisfactorily. The writer has merely attempted to place the classification of the TRYPANORHYNCHA on as satisfactory a basis as is possible in the present state of our knowledge and, in addition, has given a description, for the first time, of the anatomy of a few species.

The writer is indebted to Professor James Johnstone, Oceanographical Department, University of Liverpool, for specimens of two or three species.

## CLASSIFICATION

Rudolphi (1809) erected the genus *Tetrarhynchus* and included it in his Order **Cestoidea**; he ascribed to the genus the following characters:—

'Body flat and continuous; head furnished with two bipartite bothridia with four retractile armed proboscides.'

In the year 1819 Rudolphi erected the genus *Rhynchobothrius* to include *Bothriocephalus corrolatus* and *B. paleaceus*, but he did not define its characters.

Van Beneden (1850) included the genus *Tetrarhynchus* in his *TETRAPHILLES* and in his division PHYLLORHYNCHIENS; he defined the characters of the family as follows:—

'These animals are characterised in the first place by the four bothridia which are common to the whole section and to which correspond four retractile proboscides, armed with hooks disposed in spirals and lodged in a membranous sheath. There is always a distinct neck; the longitudinal canals traverse the entire length of the head and strobila, commencing in the interior of the bothridia.'

He referred to the genus thus:—

'As I only know one genus in this family it is useless to enumerate its characters.'

Van Beneden further stated that the genus *Tetrarhynchus* included those forms which, in the larval stage, are destitute of a vesicle, and that the genus *Rhynchobothrium* included mature worms possessing segments. As the name *Tetrarhynchus* has priority he considered the name *Rhynchobothrius* to be a *nomen nudum*.

Diesing (1850) placed the *RHYNCHOBOTHRIA* as a sub-tribe of the *BOTHRIOCEPHALIDEA* and divided it into the following genera :—

*Dibothriorhynchus* with two opposite bothridia ; body continuous.

*Tetrarhynchus* with two opposite bothridia, each bothridium being divided by a longitudinal septum.

*Rhynchobothrium* with two opposite bothridia ; body articulated.

*Tetrabothriorhynchus* with four bothridia, opposite, in pairs, apices converging.

*Stenobothrium ;* with four lateral opposite bothridia, body continuous.

*Tetrarhynchobothrium ;* with four lateral opposite bothridia ; body segmented.

*Synbothrium ;* with four terminal, cruciform, opposite bothridia joined to the head posteriorly by a membrane.

The same author in 1863 classified the Tetrarhynchids as follows :—

Section PARAMECOCOTYLEA.

Tribe *Paramecocotylea aprocta.*

Sub-tribe *Trypanorhyncha.* Head with four boring proboscides armed, and retractile into the neck.

Family DIBOTHRIORHYNCHA (characters not defined).

'Genus *Rhynchobothrium.* Body articulated ; head with two bothridia facing each other, lateral or marginal, complete and divided by a longitudinal septum ; four armed proboscides. Neck tubular ; male pore marginal, female lateral, or both marginal. In intestine of marine fishes.'

Family TETRABOTHRIORHYNCHA.

'Body articulated ; head with four lateral bothridia facing each other in pairs, or standing out terminally, disposed crosswise ; four free armed proboscides ; or singly traversing the bothridia ; neck tubular. Genital pores marginal or lateral. Parasitic in marine fishes.

Genus *Tetrarhynchobothrium.* Body articulated ; head with four lateral bothridia opposite each other in pairs ; four free proboscides. Pores marginal or lateral. Parasitic in marine fishes.

Genus *Syndesmobothrium.* Body articulated ; head with four terminal prominent bothridia placed crosswise ; four proboscides, each one running through the middle of a bothridium. Pores marginal (?). Parasitic in marine fishes.'

Carus (1863) included the genus *Tetrarhynchus,* Cuvier (?) in the sub-family 'PHYLLORHYNCHIDEA,' van Ben., of the Family 'TETRAPHYLLIDEA.' He stated that the characters of the genus were similar to those of the sub-family, viz. :—

'The scolex is separated from the strobila by a neck, and has four suckers, sometimes joined in pairs ; four proboscides always armed with hooks which can usually be drawn back into sheaths.'

In 1864, Cobbold erected the family TETRARHYNCHIDAE with characters as follows :—

'The members of this family are easily recognised by the possession of four armed retractile proboscides attached to the head. The armature consists of several successive rows of sharply-pointed, recurved hooks, frequently amounting to several thousands. The head itself is usually more or less bilobed, each half supporting either one bipartite bothrium, or else two separate fossae. These cavities are also frequently supported on four petaloid appendages, which vary much in shape in the different species, and also in the same individual, according to the degree of contraction of the part. The head and neck are continuous, and usually about the same breadth as the body, the latter being sometimes even narrower than either the head or neck. The body is depressed, filiform, distinctly segmented, and usually of great length in the mature state, the reproductive orifices being situated at the lateral margin of the joints in an irregularly alternate manner.'

Linton, 1889, sub-divided the family as shown below :—

Family TETRARHYNCHIDAE, Cobbold, 1864.

Synonyms :—Sub-tribe *Trypanorhyncha*, Diesing, 1863.
Sub-family *Phyllorhynchinae*, van Ben.

Sub-family I. DIBOTHRIORHYNCHINAE, Mont., 1892.

Synonym :—*Dibothriorhynchidae*, Dies.

Genus *Rhynchobothrium*, Rudolphi, 1819.

Synonym :—*Tetrarhynchus* of authors.

'Body taeniaeform. Neck tubular. Head continuous with neck, with two opposite bothria, parallel or converging at the apices, lateral or marginal, entire or undivided, or either bilocular with a longitudinal partition, or bilobed or divided. Proboscides four, terminal, filiform, armed, retractile in the neck, for the most part longer than the head. Genital apertures, male marginal, female lateral, or male and female marginal approximate.'

Genus *Otobothrium*, Linton, 1889.

'Body articulate, taeniaeform, head separated from body by a neck. Bothria two, opposite, lateral, each with two supplemental ciliated pits at the posterior free angles. Proboscides four, terminal, filiform, armed, retractile in neck. Reproductive apertures marginal.'

Sub-family II. TETRABOTHRIORHYNCHINAE, Mont., 1888.

Synonym :—*Tetrabothriorhynchidae*, Dies., 1863.

Genus *Tetrarhynchus*, Rudolphi, 1809.

Synonyms :—*Bothriocephali* spec., Bartels ?
*Rhynchobothrii* spec., van Ben. and R. Leuckart.
*Tetrarhynchi* spec., van Ben.
*Aspidorhynchus*, Molin, 1858.
*Tetrarhynchobothrium*, Dies.

'Body articulate, taeniaeform. Neck tubular. Head with four bothria in two lateral pairs, parallel with the head. Proboscides four, terminal, filiform, armed, retractile in the neck, free, *i.e.*, not running through the bothria. Genital apertures marginal or lateral.'

Genus *Syndesmobothrium*, Diesing, 1854.

'This genus is characterised by Diesing as follows :—Body articulate taeniaeform ; neck tubular, rounded at the base ; head tetragonal, with four terminal prominent bothria attached to head by posterior margin, cruciformly disposed, oval, slightly convex, joined with each other at the base by a membrane ; proboscides four, filiform, armed, each one running through a bothrium (pedicel) excurrent at apex, long, retractile in the neck. Genital apertures marginal (?). In the intestines of marine fishes of tropical America.'

Loennberg, in 1889, erected the COENOMORPHINAE as a sub-family of the TETRARHYNCHIDAE. Type and only species *C. grossus* (Rud.) = *T. linguatulus* (van Ben.) = *T. solidus*, Drummond, 1838. Larvae have been recorded in Decapods.

The principal characters of the sub-family are :—(1) the presence of a double set of genitalia in each segment, and (2) the fact that the worms are very stout and muscular.

Braun (1900) defined the Order **Trypanorhyncha,** Diesing, 1863, as follows :—

'The scolex is divided into head and neck ; head with two or four bothridia and with four retractile and armed proboscides ; segmentation complete, segments usually dividing off before maturity. Pores marginal or sub-marginal, uterine pore ? Genitalia as in TETRAPHYLLIDEA. Larvae in different marine animals, adults usually in the guts of Plagiostomes.'

He placed the following genera in the family ; an abstract of his definitions is given below :—

*Rhynchobothrius*, Rud.

This is the oldest genus, erected to accommodate the species *Bothriocephalus corollatus* and *B. paleaceus*, Rud. The author only saw drawings of these species. He discusses the question as to whether *B. corollatus* and *Taenia corollata*, Fab., are synonymous, but says too little is known to decide. *R. corollatus* is selected as the type species. It has two simple bothridia with four proboscides at the four corners, each proboscis being furnished with 20 to 30 hooks bent posteriorly.

*Dibothriorhynchus*, de Blainv., 1824 (as appendix to French translation of Bremser).

The drawing shows an unsegmented cestode with a posterior tubercle, two bothridia with a posterior longitudinal septum, two short, retractile proboscides armed with small, bent hooks. Later on the worm was named *D. lepidopteri*. The author considers two proboscides a mistake of the observer, or else, according to Loennberg, it was a larval form. Loennberg's observations enable the following amended description to be given :—

'TRYPANORHYNCHA with four, short, thick, club-shaped or half-circular retractile proboscides armed with hooks ; and two sessile, powerful suckers ; segmentation complete ; segments very muscular, always broader than long, not dividing off ; genitalia double in each proglottis, each set having three pores ; cirrus and vagina marginal, uterus ventral. Larvae not encysted ; adult in stomach of Selachiens.'

Diesing set up another genus *Dibothriorhynchus* for *Tetrarhynchus scolecinus*, Rud., and *T. gracilis*, Rud. (*i.e.*, larval forms), but adult forms have also received the name ; they really belong to the genus *Rhynchobothrius*. Thus Diesing's *Dibothriorhynchus* is a synonym of *Rhynchobothrius*.

*Tetrarhynchobothrium*, Dies. Type species *T. tenuicolle*, Diesing, but four other species were added to this genus later on.

Characters of the genus are :—Cylindrical neck, four bothridia, four thread-like proboscides, and irregularly alternating marginal pores ; ripe segments longer than broad. *Tetrabothriorhynchus*, Dies., is a synonym of this genus.

*Aspidorhynchus*, Mol.

Contains one species only, viz., *A. infulatus*, Mol. Diesing placed it in the genus *Tetrarhynchobothrium*. Molin's characteristics include the form and position of the bothridia and a telescopic, retractile neck ; these characters are not sufficient to justify the retention of genus *Aspidorhynchus*.

*Synbothrium*, Diesing. Diesing later on changed the name to *Syndesmobothrium*.

Characters :—Four oval, convex bothridia placed crosswise on the surface of the head and bound together by a basal membrane ; four, thread-like proboscides, which traverse the longitudinal axes of the bothridia and emerge anteriorly. Genital pores marginal. *Synbothrium fragile*, Dies., was for a long time the only species, but Linton described *S. filicolle*, though he has not described it sufficiently, as the specimen was not mature. Host :—*Trygon centrura* (spiral valve).

*Abothros*, Welch. Only species :—*A. carcharias*, Welch. from *Carcharias*, sp.

Chief characters :—Alleged absence of bothridia ; the four, slender proboscides are armed with hooks bent backwards, emerging close together on the ventral surface of the head. Segments very short. Genital pores ? Certain longitudinal furrows could be seen on the scolex, probably the missing bothridia. *A. carcharias*, Welch, is probably identical with *Bothriocephalus bicolor*, v. Nordm.

*Otobothrium*, Linton. The only species is *O. crenacolle*, Linton.

Characters :—Two ventral bothridia with longitudinal septum, each of which bears on its free posterior edge, two little suckers. Four, thread-like proboscides, pores marginal.

Lühe (1910) defined the Order **Trypanorhyncha** thus :—

'Cestodes whose scolex is usually continued into a *Kopfstiel* ; with two or four bothridia at whose apical end are four armed extensile proboscides. When retracted (with the assistance of a retractor which runs in their interior and is inserted into their anterior end) each is drawn back into a proboscis sac. This corresponds in thickness and length with the proboscis itself and represents a direct continuation of the proboscis into the anterior end of the scolex and *Kopfstiel*. At its inner end, the sheath passes directly into the visibly thicker, sharply delineated, egg-shaped or sausage-shaped sac, whose contraction brings about the extrusion of the proboscides. Outer segmentation complete. Formation of segments as in TETRAPHYLLIDEA ; mature in stomach or spiral valve of Selachiens ; larvae found in all kinds of marine animals. In fresh water only a few species are found in the larval condition as parasites of Teleosts. No details of the deveploment of the larvae are known.'

Lühe distinguished two families, *viz.* :—

1. Larva encysted ; proboscis long, slender, cylindrical, whole body not massive or muscular..... *Tetrarhynchidae*, Lühe, 1910
2. Free larvae, not encysted ; probocsis short ; almost semi-globular or club-shaped ; whole body robust and muscular..... *Coenomorphidae*, Lühe, 1910

He ascribed the following characters to the TETRARHYNCHIDAE :—

'Scolex with long, slender, cylindrical, very mobile proboscides, with two or four very mobile bothridia more or less leaf-like. *Kopfstiel* present. Strobila slender, with little muscular development ; often transparent. Segments, when mature, longer than broad, easily detachable ; in each segment a single set of genital organs. Uterus apparently without primary pore. Ripe eggs, as in TETRAPHYLLIDEA, escape through dehiscence. In spiral valve of Selachiens ; larvae in Turtles, Bony fish, Cephalopods and Decapods.'

He added that nothing was known regarding the systematic division of the family.

The family COENOMORPHIDAE, he defined as follows :—

'Scolex very robust with short, thick proboscides, semi-globular or club-shaped, with two simple bothridia sunk into the scolex like a pit or a split, and with edges which hardly protrude ; no *Kopfstiel*. Strobila robust and very muscular, up to 4 mm. in thickness and not transparent ; segments when mature much broader than long and not separating off. In each segment there are two sets of genital organs ; uterus with a special pore opening ventrally and having its own muscular system. Mature in stomach of sharks ; larvae, not encysted, found in bony fish. There is only one genus with one species, viz., *C. grossus* (Rud.) = *T. solidus* = *T. linguatulus*.'

Pintner (1913) in dealing with Tetrarhynchids in general, pointed out that our information relating to the anatomy of the various forms was not sufficient to enable one to deal extensively with the order, as only six species appear to be well-determined, *T. ruficollis*, Eysenh., 1829, being the best known. Specific points are hardly ever mentioned by authors who have hitherto described different species. The principal characters in distinguishing species, according to Pintner, are :—

(1) The scolex ; but this is not altogether definite ; the length of the part of the bothridium attached to the head is of importance, the peduncle behind the head is included as scolex. (2) The exact number and shape of hooks. (3) The form of the proboscis sacs. (4) Whether the head is separated from the neck. (5) The general appearance of the worm. (6) The specific characteristics of ripe segments. (7) The presence or absence of a uterine pore.

Pintner identified three groups, viz. :—

1. With a true uterine pore present ..... *T. viridis* group
2. With an involuted, apparent pore, not found in anterior segments ..... *T. ruficollis* group
3. Segments dehiscent, no uterine pore either primary or secondary ..... *R. tenuis* group

He defined a number of genera as follows :—

*Eutetrarhynchus*, Pintner, 1913.

'Scolex very long and slender, with small proboscides. The two flat, spoon-like bothridia are deeply embedded (in lateral view) and inclined to the long axis at about 45°. Proboscis bulb at least twice as long as the rest of the scolex ; in this respect it resembles *T. longicollis*. The proboscides are very long and thick and are covered with a pile of small uniform hooks which look black in low magnifications owing to their small size, but this is not so when highly magnified. Proboscis not longer than proboscis sheath. The retractor lies in the fundus of the *Kolben* (knob) and is formed of parallel, closely arranged fibres in which the large constituent cells, for their whole length, and in large numbers, are arranged on one side. The very long knobs are formed of only five or six broad shells of clearly striped, transverse muscle bands, in a single layer, with large spherical myoblasts arranged on the inner side of the knobs in the form of stripes. Neck short ; chain weak but markedly craspidot, seldom apolytic. Generally one or two very mature segments, or longer pieces of greater age, become detached from the chain. Frequently they retain the primary end segment. Segments at the end of the chain sometimes longer than broad. Genital pore in the middle of the margin ; apparent uterine openings in the middle line on a level with the pore. The oviduct opens far in front into the uterine sac. Testes large and very numerous, occupying all the segment. Type species :—*Eutetrarhynchus ruficollis* (Eysenhardt, 1829). *E. leucomelanus*, Shipley and Hornell, 1906, also belongs to the same genus.'

*Stenobothrium*, (Diesing, 1863).

'Four bothridia (two dorsal and two ventral) whose sides are furnished with very long (but not projecting) hairs ; proboscides very long. In *S. macrobothrium* they are the longest of any known *Tetrarhynchid*, being several times as long as the rest of the cephalic region. Scolex markedly craspidot ; proboscides weak, short, thin and thread-like, with uniform, not numerous, small hooks, rather far apart. The sheaths are correspondingly thin and short and they bear small knobs. The knobs are formed of very numerous thin muscles in six shells, each of which consists of many muscle layers, the innermost being the thinnest. In transverse section the knob muscles appear not to be arranged as usual, but to lie parallel like four horses racing side by side, the thinnest part of each muscle representing the head and the thickest part the hind end of the horse ; retractor one-celled. A single, large, constructive cell occurs between the fibrillae, and these have no nuclei ; their surface is covered with minute (chitin-like ?) papillae. Frontal glands powerfully built. Strobila strongly developed. Even the last segments broader than long, with completely smooth sides, laterally limited by a straight line. Genital cloaca situated at the anterior end of the segment towards the ventral side ; the long cirrus sac stretches the length of the anterior margin of the segment, its end being bent backwards. Uterine openings in the middle line behind one another. Vitelline glands not so dense as the testes generally are, because the ventral sides are occupied by the coils of the uterus. Ovary of two dorsal and two ventral wings, which, on each side, are sub-divided into five or six lobes ; the tubes connecting these sub-divisions are very thin and the wall consists of a single layer of cells ; a muscular septum is present at the end of each segment. At the primary hind end (especially in the larva) there projects a conical knob from inside the bladder which is thickly covered with strong hairs, (viz., *S. macrobothrium*) or with papillae (viz., *S. linguale*). Type species :—*S. linguale*, Cuvier, 1817. Other species :—*S. macrobothrium* (Rudolphi, 1819), *T. bisulcatum*, Linton, 1889, *T. tenue*, Linton, 1890, *T. robustum*, Linton, 1890, *S. herdmani*, Shipley and Hornell, 1906, *S. perideraeum*, Shipley and Hornell, 1906. One of Linton's species is probably identical with *S. linguale* Cuvier, 1817.'

*Lakistorhynchus*, Pintner, 1913.

'Head small and fragile; delicate, fine, and very pointed angle-shaped hooks occur on the soft thin proboscides. Strobila euapolytic or hyperapolytic; the segments grow a lot after separation. They are very long and, in the chain, pass very suddenly from unripe to ripe. The genital atrium is situated in the middle of the segment and is distinguished by a sucker-like pit in front and behind it. The surface of the ripe segments bears marked papillae. The proboscis sheath affords characters which are peculiar in two ways. Whereas in *benedeni* they are long and form spirals (in connection with a very contractile pars vaginalis) so that the proboscides, even in a completely withdrawn condition, do not approach by a long way the muscle knobs; in *platycephalus* and *rubromaculatus*, on the contrary, the sheaths are short and, therefore, also not in spiral, and the proboscides when fully drawn in, actually extend from the part bearing the hooks to the middle of the hollow space of the muscle knobs; consequently, the retractor in them is arranged in very dainty stripes. This divergence in scolex structure can easily be used as of generic importance. At times, also, the species are very peculiar. On the other hand there is between *benedeni* and *platycephalus* such a similarity in proglottides, and in the case of *benedeni* and *rubromaculatus* such a correspondence in the scolex that I must, for the present, place them in the same genus. Type species:—*L. benedeni*, Crety, 1890. Other species:—*L. rubromaculatus*, Diesing, 1863, *L. platycephalus*, Shipley and Hornell, 1906.'

*Halsiorhynchus*, Pintner, 1913.

'Probably one has to make a new genus for Shipley and Hornell's *T. ruficollis*, type species *H. shipleyanus* as the chief characters are the proboscis hooks and the "coat of mail." It is like a species I described as *Rhynchobothrius vario-uncinatus*. Shipley and Hornell's form has not the slightest resemblance to *T. ruficollis* (Eysenhardt, 1829). The chief characteristic of Shipley and Hornell's species is the armoured chain of proboscides hooks.'

*Sphyriocephalus*, Pintner, 1913.

'Species thick and muscular; two large, sucker-like bothridia, one dorsal and one ventral, giving the head a hammer-like appearance. Extruded proboscides rigid, thick and straight; proboscis sacs transverse; anterior extremity pointing outwards. The *attenuatus*-group shows the same character, which is also common in the larva, but in the larvae the posterior end of the sacs turns outward. Scolices craspidot; hooks almost equal in size and in 20 transverse and 16 longitudinal rows. The little hooks measure 0·009 mm. and 0·02 mm., the hooks are slightly larger in the middle, viz., 0·045 to 0·075 mm.; bases 0·048 mm. Eggs in one species are bell-shaped with two enormous processes at the poles, one is long, the other is small (47 to 65$\mu$ long and 6$\mu$ broad at base) and claw-shaped. Egg 75$\mu$ × 47$\mu$, one side being flattened. Type species:—*S. viridis*, Wagener, 1854. Another species:—*S. turgistensis*, Pintner, 1913.'

*Attenuatus* group.

Pintner did not name or define the group, but he included in it the following:—

'Genus *Coenomorphus*, Loenn., 1889; also *T. attenuatus*, Rudolphi, 1819, *T. grossus* (Rudolphi, 1819) and *T. megalocephalus*, Shipley and Hornell, 1906.

'*T. equidentatus*, Shipley and Hornell, 1906, also possibly belongs to the *attenuatus* group, or it is a transitional form between the *attenuatus* group and *Stenobothrium*.'

*Otobothrium*, Linton, 1890.

'Type species *O. crenecolle*, Linton, 1890. *T. carcharidis*, Shipley and Hornell, 1906, is possibly synonymous with the type species.'

Pintner gave the following synonymy for the species mentioned below :—

*Tetrarhynchus leucomelanus*, Shipley and Hornell, 1906 = *Eutetrarhynchus leucomelanus.*
,, *herdmani*, Shipley and Hornell, 1906 = *Stenobothrium herdmani.*
,, *tenue*, Linton, 1890 } = All *Stenobothrium* sp.
,, *bisulcatum*, Linton, 1889 } = All *Stenobothrium* sp.
,, *robustum*, 1890 } = All *Stenobothrium* sp.
,, *platycephalus*, Shipley and Hornell, 1906 = *Latistorhynchus platycephalus.*
,, *benedeni* = *T. tenius* = *T. gracilis* = *L. benedeni.*
,, *ruficollis*, Shipley and Hornell, 1906 = *Tetrarhynchus shipleyanus* = *Halysiorhynchus shipleyanus* = *Rhynchobothrius vario-uncinatus.*

The same author adopted the terminology noted below :— The head is *craspidot* where there is a division between the head and the neck. It is *acraspidot* where the division is absent. Ripe segments are *anapolytic*, when they remain attached to the strobila. Ripe segments are *apolytic* when they automatically separate from the strobila. Gravid segments are *euapolytic* when they separate from the chain and continue to grow. Gravid segments are *hyperapolytic* if they separate from the strobila before they are mature and especially if they do so before the uterus is developed.

The table given below summarises the details relating to the bothridia in the different genera according to the authors noted below.

Until more material is available for examination the writer proposes the following classification.

Order **Trypanorhyncha,** Diesing, 1863, emended.

Family I. COENOMORPHIDAE, Lühe, 1910, emended.
Genus *Coenomorphus*, Loennberg, 1889, emended.

Family II. TETRARHYNCHIDAE, Cobbold, 1864, emended.
Genera :—*Tetrarhynchus*, Rud., 1809, emended.
*Rhynchobothrius*, Rud., 1819, emended.
*Syndesmobothrium*, Diesing, 1863.
*Otobothrium*, Linton, 1890.

The characters of the order, families and genera are given below.

TABLE I

Table of Genera

| Author | Genus | Bothridia |
|---|---|---|
| Rudolphi, 1809 ... | *Tetrarhynchus* ... ... ... | Two bothridia; bipartite |
| Rudolphi, 1819 ... | *Rhynchobothrius* ... ... ... | Two simple bothridia |
| Blainville, 1824 ... | *Dibothriorhynchus* ... ... ... | Two simple bothridia |
| Van Beneden, 1849 | *Tetrarhynchus* ... ... ... | Four bothridia |
| Diesing, 1850 ... | *Dibothriorhynchus* ... ... ... | Two opposite bothridia; body continuous |
| Diesing, 1850 ... | *Tetrarhynchus* ... ... ... | Two opposite bothridia, each bothridium being divided by a longitudinal septum |
| Diesing, 1850 ... | *Rhynchobothrium* ... ... ... | Two opposite bothridia; body articulated |
| Diesing, 1850 ... | *Tetrabothriorhynchus* ... ... | Four bothridia, opposite; in pairs apices converging |
| Diesing, 1850 ... | *Stenobothrium* ... ... ... | Four lateral opposite bothridia; body continuous |
| Diesing, 1850 ... | *Tetrarhynchobothrium* ... ... | Four lateral opposite bothridia; body segmented |
| Diesing, 1850 ... | *Synbothrium* ... ... ... ... | Four terminal cruciform opposite bothridia joined to head posteriorly by a membrane. |
| Diesing, 1863 ... | *Rhynchobothrium* ... ... ... | Two bothridia divided |
| Diesing, 1863 ... | *Tetrarhynchobothrium* ... ... | Four bothridia |
| Diesing, 1863 ... | *Syndesmobothrium* (*Synbothrium* renamed) | Four terminal bothridia |
| Linton, 1889 ... | *Rhynchobothrium* (= *Tetrarhynchus* of authors) | Two bothridia; entire or sub-divided |
| Linton, 1889 ... | *Otobothrium* ... ... ... ... | Two bothridia with posterior ciliated pits |
| Linton, 1889 ... | *Tetrarhynchus* ... ... ... | Four bothridia |
| Linton, 1889 ... | *Syndesmobothrium* ... ... ... | Four terminal bothridia |
| Braun, 1900 ... | *Rhynchobothrius* ... ... ... | Two bothridia |
| Braun, 1900 ... | *Dibothriorhynchus* ... ... ... | Two bothridia each with a septum |
| Braun, 1900 ... | *Tetrarhynchobothrium* ... ... | Four bothridia |
| Braun, 1900 ... | *Synbothrium* = *Syndesmobothrium* ... | — |

It will be seen from the above table that the genus *Tetrarhynchus*, as originally described by Rudolphi, had two bipartite bothridia and that he applied the name *Rhynchobothrius* to forms with two simple bothridia.

Van Beneden was the first to state definitely that the genus *Tetrarhynchus* possessed four bothridia, although the fact is implied in Rudolphi's definition of the genus.

The synonymy of the two genera is therefore as follows :—

*Tetrarhynchus*, Rudolphi, 1809.

Synonyms :—*Tetrarhynchus*, van Ben., 1849.
*Tetrarhynchus*, Diesing, 1850.
*Tetrabothriorhynchus*, Diesing, 1850.
*Stenobothrium*, Diesing, 1850.
*Tetrarhynchobothrium*, Diesing, 1850 and 1863.
*Rhynchobothrium*, Linton, 1889, pro parte.
*Tetrarhynchus*, Linton, 1889.
*Dibothriorhynchus*, Braun, 1900.
*Tetrarhynchobothrium*, Braun, 1900.

*Rhynchobothrius*, Rudolphi, 1819.

Synonyms :—*Dibothriorhynchus*, Blainville, 1824.
*Dibothriorhynchus*, Diesing, 1850.
*Rhynchobothrium*, Diesing, 1850.
*Rhynchobothrium*, Linton, 1889, pro parte.
*Rhynchobothrius*, Braun, 1900.

Although it is easy to refer worms with two or four bothridia to their respective genera, there are a few species in which each bothridium is only *partially* divided and it may then become difficult to decide whether there are two or four bothridia. Such forms are to be regarded as intermediate and may be referred to either genus.

Characters of the Order **Trypanorhyncha,** Diesing, 1863, emended.

Head with two or four bothridia, and bearing four retractile proboscides armed with hooks ; segmentation complete. Genital organs as in the *TETRAPHYLLIDEA*. Adult in marine Elasmobranch fishes and occasionally in Teleosts. Larvae in Teleosts and invertebrates. With two families.

Family I. TETRARHYNCHIDAE, Cobbold, 1864, emended.

**Trypanorhyncha** with a single set of genitalia in each segment. Worms more or less fragile. Parasitic in marine and fresh water fishes.

Family II. COENOMORPHIDAE, Lühe, 1910, emended.

**Trypanorhyncha** possessing a double set of genitalia in each segment ; strobila stout and muscular. Parasitic in marine and fresh water fishes.

KEY TO GENERA

A single set of genitalia in each segment.....1
A double set of genitalia in each segment..... *Coenomorphus*

1. Head with four bothridia.....2
Head with two bothridia.....3

2. Bothridia parallel with body and with posterior half free..... *Tetrarhynchus*
   Bothridia terminal, cruciform, at right-angles to body; attached to head by posterior margin..... *Syndesmobothrium*
3. Bothridia with ciliated pits at their posterior margin..... *Otobothrium*
   Bothridia without ciliated pits; each bothridium often more or less divided into two..... *Rhynchobothrius*

Genus *Tetrarhynchus*, Rudolphi, 1809, emended.

*Head with four bothridia arranged in pairs, and lying parallel with the head.*

*Tetrarhynchus tetrabothrius* (van Ben., 1849).

Several specimens from *Acanthia vulgaris*, North Sea, August, 1923, collected by the author; also from the dog-fish, Isle of Man, collected by Mr. Birtwistle.

*Tetrarhynchus perideraeus*, Shipley and Hornell, 1906 (figs. 1 to 3).

This species, according to Shipley and Hornell, measures up to 70 mm. in length and 1·3 mm. in breadth. The head bears two lappets, but they are so divided in the centre as to appear like four; the proboscides are slender and bear oblique rows of very minute teeth all of uniform size; the proboscis tubes and proboscis sheath

FIG. 1. *Tetrarhynchus perideraeus.* A hook. × 1125.

FIG. 2. *Tetrarhynchus perideraeus.* A row of hooks on proboscis. × 500.

are also short. The head is produced backwards into a very characteristic collar which overlaps and embraces the anterior part of the body. The neck is fairly long; the segments have straight sides, except posteriorly; the middle portion of the strobila shows a

tendency to become coiled or twisted ; the genital pores are irregularly alternate and are situated about the middle of the lateral margin. The species was obtained from the intestine of *Carcharias gangeticus*.

The writer obtained a few specimens of this species from the intestine of *Ginglymostoma concolor*, Pearl Banks, Ceylon, 27th February, 1909, and adds the following notes on the anatomy of the species :—

External Anatomy. The *head* measures about 1·36 mm. in length and 0·85 mm. in breadth ; the hooks are spirally arranged and measure 16$\mu$ ; neck absent. There are a large number of segments having convex margins ; the genital pores are lateral and irregularly alternate, being situated a little in front of the centre.

Internal Anatomy. *Testes*. The testes vary in number from 60 to 70 ; they occupy the entire dorsal area within the

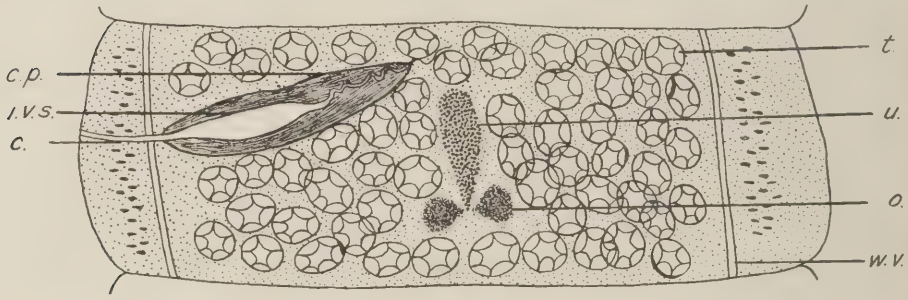


Fig. 3. *Tetrarhynchus perideraeus*. Mature segment. *t.*—testes ; *u.*—uterus ; *o.*—ovary ; *w.v.*—water vessel ; *c.*—cirrus ; *i.v.s.*—internal vesicula seminalis ; *c.p.*—cirrus pouch. × 112.

excretory vessels and a few testes are situated posterior to the ovary.

*Vas deferens*. The cirrus pouch is conspicuous and lies anterior to the vagina and median to the excretory vessels ; it communicates with the exterior by means of a long narrow duct. In the median direction it extends almost half-way across the segment, its median extremity being closely apposed to the anterior extremity of the segment ; no spines were observed on the cirrus. The vas deferens lies coiled within the cirrus pouch, near the median extremity of which it dilates into a seminal vesicle.

*Ovary*. This is peculiar in being situated some distance from the posterior extremity of the segment, and in being small and dumb-bell

shaped ; it stains very deeply and the two lobes are very compact. The vagina runs posterior to the cirrus pouch.

*Vitelline glands.* These are very scanty and consist of single acini practically encircling the segment.

*Uterus.* This consists of a sac with irregular walls which completely fills the segment ; it was full of immature eggs.

### *Tetrarhynchus aetobatidis*, Shipley and Hornell, 1906.

One specimen, immature, was collected by the author from the intestine of *Trygon kuhli*, Ceylon Pearl Banks, 1909.

This species is easily identified on account of the fact that (1) the head is swollen, the swelling being due to the stout muscular proboscis sacs ; (2) there is a deposit of pigment (present in preserved specimens) at the junction of the proboscis tubes with the proboscis sacs.

As the specimen was immature, the anatomy is not known.

### *Tetrarhynchus* sp.

The larval form of the species about to be discussed occurs in the Ceylon Pearl Oyster and was originally believed to be concerned in pearl production. The writer has recently (1924) summarised our knowledge relating to the pearl-inducing parasite in the Ceylon pearl oyster, except with regard to the *Tetrarhynchus* larva. At least three different species of larval cestodes inhabit the tissues of the pearl oyster. Two of these are globular and belong to the genus *Tylocephalum*, whilst the third is elongated and belongs to the genus *Tetrarhynchus*. There can be no doubt that the larval parasites concerned in pearl production belong to the former genus ; the larval Tetrarhynchid is confined almost exclusively to the gut wall of the oyster ; Shipley and Hornell (1904) described the adult of this larva as occurring in *Rhinoptera javanica*, and they named the adult worm *Tetrarhynchus unionifactor*. All the three larval forms found in the pearl oyster were believed by Shipley and Hornell, without sufficient reason, to be young forms of the adult *Tetrarhynchus unionifactor* found in *Rhinoptera javanica*. For reasons given by the writer in the paper referred to above, the specific name *unionifactor* is now restricted to the adult form of the larger globular larva found

in the pearl oyster, which is now known as *Tylocephalum unionifactor*; in all probability *Tylocephalum dierama*, Shipley and Hornell, 1906, is a synonym of *T. unionifactor*. The Tetrarhynchid larva found in the pearl oyster is therefore without a name.

As a result of feeding sharks and rays with infected pearl-oysters the writer, on two occasions, obtained large numbers of an adult Tetrarhynchid, which, at the time, he believed to be identical with *Tetrarhynchus unionifactor*, Shipley and Hornell, 1904.

Unfortunately, nearly all the material has been lost and it is therefore impossible to describe the adult worm until more material is available. The adult worms obtained by the writer differ, however, from the worm described by Shipley and Hornell in having four instead of two bothridia, and in the hooks not being all alike as they are stated to be in Shipley and Hornell's worm.

Professor Th. Pintner, of Vienna, has been good enough to examine the fragments of adult worms obtained by the writer from *Ginglymostoma concolor*, as a result of feeding experiments, and he is of opinion that the species belongs to his genus *Latistorhynchus* because (1) the head is small and delicate, (2) the proboscides are long, the hooks fine and pointed, and (3) the proboscis sheaths are spiral. The scarcity of material did not allow of a definite identification, but the species is close to, but different from *L. benedeni*, Crety, 1890. Shipley and Hornell state that in their larval form of *Tetrarhynchus unionifactor*, the body is covered with warts and this feature is, according to Pintner, one of the characteristics of the genus *Latistorhynchus*. It is quite possible that Shipley and Hornell's species, and that obtained by the writer, belong to the same genus, but they are certainly different species, and the writer's specimens are closely related to, if not identical with, *Tetrarhynchus rubromaculatus* (Diesing), Shipley and Hornell, 1906.

### Genus *Rhynchobothrius*, Rud., 1819, emended.

*Body taeniaeform. Head with two lateral or dorso-ventral bothridia, each bothridium being either entire, or partly divided.*

*Rhynchobothrius erinaceus* (van Ben., 1858).

SYNONYMS :—*Rhynchobothrium imparispine*, Linton, 1890.
*Rhynchobothrium simile*, Linton, 1909.
*Tetrarhynchus gangeticus*, Shipley and Hornell, 1906.
*Tetrarhynchus annandalei*, Hornell, 1912.

Very numerous specimens from the spiral valve of *Trygon* sp., Ceylon Pearl Banks, 1910. Collected by the author.

Shipley and Hornell, in 1906, under the name *T. macroporus*, described a worm which appears to differ from *R. erinaceus* only in having the bothridia

'Each divided into two, each half corresponding with one of the four hooked proboscides.'

The writer has had the opportunity ot examining the type (and only) specimen of *R. annandalei* obtained from *Stegostoma tigrinum*.

The proboscides were not extruded and the shape of all the hooks could not be determined ; it was noted, however, that they were of various shapes, many of them being large. The testes are numerous and the pores are irregularly alternate, large, and situated in the posterior third of the segment. The worm is indistinguishable from *R. erinaceus*.

*Rhynchobothrius macrocephalus* (Shipley and Hornell, 1906) (fig. 4).

SYNONYMS :—*Tetrarhynchus macrocephalus*, Shipley and Hornell, 1906.
*Tetrarhynchus ruficollis*, Shipley and Hornell, 1906.

Numerous specimens from the intestine of *Rhynchobatus djeddensis*, Ceylon Pearl Banks, February 3rd, 1911 ; also from *Trygon walga*, November 27th, 1910. Collected by the author.

Shipley and Hornell described this species from a young form which was quite immature, and which measured 8 mm. only in length. The head measured 6 mm. in length and the rest of the body 2 mm. They called attention to the fact that the distinctive character of this species was

'The herring-bone spicules on the proboscides and the grading of the hooks on the same.'

Pintner, who examined the type species, states that *T. macrocephalus* is the same as the mature worm measuring 40 mm. to 50 mm., identified by Shipley and Hornell, as *T. ruficollis* (Eysenhardt, 1829), and with this statement the present writer is in agreement. Pintner

further points out that the worm identified by Shipley and Hornell as *T. ruficollis* (Eysenh.) is quite a different species from that described by Eysenhardt.

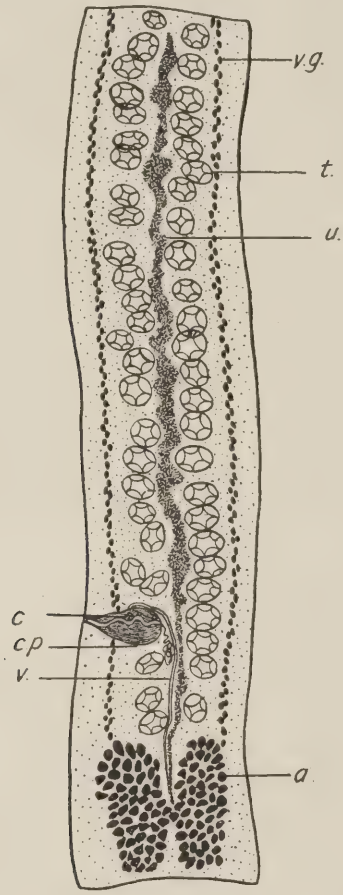


FIG. 4. *Rhynchobothrius macrocephalus.* Mature segment. *v.g.*—vitelline glands; *t.*—testes; *u.*—uterus; *c.p.*—cirrus pouch; *c.*—cirrus; *o.*—ovary; *v.*—vagina. × 69.

Pintner erects a new genus which he names *Halsiorhynchus* to accommodate Shipley and Hornell's *T. macrocephalus* and he also changes the specific name to *shipleyanus*.

For reasons which are stated elsewhere, the writer is of opinion that Pintner's classification cannot be accepted until more is known regarding the morphology of the **Trypanorhyncha** generally, and

further, the changing of the specific name to *shipleyanus* is clearly contrary to the accepted rules of nomenclature. Pintner states that

'One has to make a new genus for Shipley and Hornell's *T. ruficollis* (type species *Halsiorhynchus shipleyanus*) as the chief characters are the proboscis hooks some of which are arranged like " a coat of mail or armoured chain " (herring-bone type). It is like a species I described as *Rhynchobothrius vario-uncinatus*. Shipley and Hornell's form has not the slightest resemblance to *T. ruficollis* (Eysenh.).'

As *T. macrocephalus* was described first, and as it is identical with the worm which Shipley and Hornell called *T. ruficollis* (Eysenh.), it is clear that the specific name *macrocephalus* must stand.

As the anatomy of the worm has not been described, the following notes are now added :—

The worms measure up to 50 mm. in length ; there is nothing to add to the description of the head except that there are two bothridia only. There is no neck. The worm is composed of about thirty-five segments and the genital pores are irregularly alternate and situated in the posterior third of the segment. There are about fifty-five testes ; the *cirrus pouch* extends almost to the median longitudinal axis of the segment. The *ovary* is bilobed, each lobe consisting of about fifteen large acini. The *oviduct* is short and appears to run anterior to the pouch. In segments mounted entire, the *vitelline glands* appear to consist of a single row of acini running along the lateral margins, but they actually encircle the entire segment. The *uterus* at first consists of an irregular tube running in the median antero-posterior axis of the segment. It eventually becomes bag-shaped and entirely fills the segment.

### *Rhynchobothrius rhynchobatidis*, Shipley and Hornell, 1906 (figs. 5 to 9).

Synonyms :—*Tetrarhynchus rhynchobatidis*, Shipley and Hornell, 1906.
*Rhynchobothrium curtum*, Linton, 1909.

Three specimens from the intestine of *Trygon sephen.*, Ceylon Pearl Banks ; collected and presented by James Hornell, Esq., F.L.S.

External Anatomy. The worms are relatively large and stout, measuring up to 50 mm. in length and having a maximum breadth of 1·5 mm. They are composed of numerous thick segments, with slightly salient posterior margins, the last segment measuring 2 mm. in length and 1·4 mm. breadth ; the genital pores are irregularly alternate and are situated in the posterior third of the segment.

*Head.* The head is very small and somewhat heart-shaped, the pointed extremity being directed anteriorly. It has a maximum width of 750$\mu$ and a length of 850$\mu$. The two bothridia are small, having a breadth of 530$\mu$ and a length of 470$\mu$; their margins are entire and only slightly thickened. The proboscis sacs are situated almost immediately behind the posterior margins of the bothridia and they have a length of 245$\mu$ and a maximum breadth of 110$\mu$. The neck measures about 220$\mu$ in length; anteriorly it is somewhat thickened and the posterior extremity of the proboscis sacs lie in the thickened portion. The armed portion of the proboscides and the proboscis sheaths are each about as long as the bothridia.

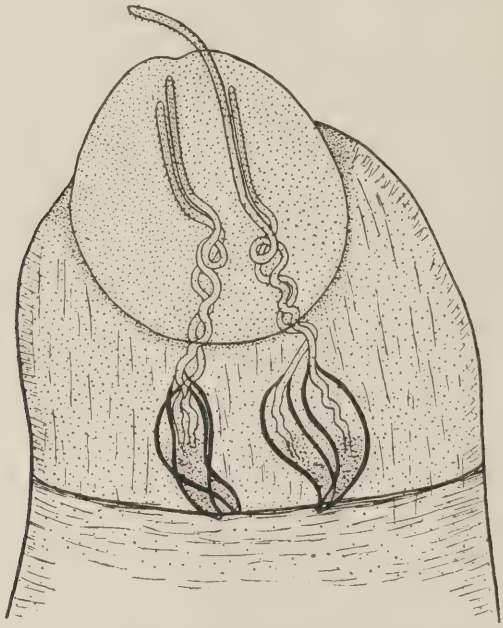

Fig. 9. *Rhynchobothrius rhynchobatidis.* Head. × 69.

*Hooks.* The hooks on the proboscides appeared to be arranged irregularly and not, as is usual, in rings or in spirals. The internal face of each proboscis bears a longitudinal row of rose-thorn shaped hooks 8$\mu$ in length arising from a base also 8$\mu$ in length. Ventrally there are three or four longitudinal rows of smaller rose-thorn shaped hooks, two longitudinal rows of which have their points directed anteriorly instead of posteriorly; in Shipley and Hornell's specimens,

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FIGS. 5 to 8. *Rhynchobothrius rhynchobatidis.* Hooks from various parts of proboscis. × 1125.

only one longitudinal row of hooks pointed anteriorly. Dorsally there are two more rows of small rose-thorn shaped hooks gradually changing in shape both dorsally and ventrally into about two longitudinal rows of very slender, sabre-like hooks, also 8$\mu$ in length on the external face of each proboscis. The neck is very short, measuring only 200$\mu$ in length.

INTERNAL ANATOMY. *Muscular system.* The longitudinal muscular system is strongly developed and consists dorsally and ventrally of a single row of oval bundles. Laterally the bundles are much smaller and scattered about irregularly.

*Excretory system.* This consists of two vessels on each side, the internal vessel is large and the external vessel (which lies directly external and close to the large vessel) is very small.

Details of the nervous system were not investigated. The cortical and medullary parenchyma is strongly developed.

*Genitalia. Testes.* These are very numerous and fill the dorsal part of the segment in front of the ovary. In the early stages of development they are crowded together in the median field, on each side of the mid-antero-posterior axis. The cirrus pouch is conspicuous and extends one-third the distance across the segment; no spines were observed on the cirrus. Posterior and median to the pouch the vas deferens forms a number of conspicuous coils.

*Ovary.* This is, as usual, a bilobed organ situated posteriorly; the vagina is a short, coiled tube which runs ventral (?) to the pouch and opens to a shallow genital atrium.

*Shell gland.* This is a conspicuous organ lying posterior to the ovary.

*Vitelline glands.* These completely encircle the segment and are situated in the cortical parenchyma.

*Uterus.* This arises as a closely coiled tube running to the extreme anterior margin of the segment; eventually it entirely fills the segment, and is distended with eggs, none of which, however, were mature.

DIAGNOSIS. This species resembles *T. rhynchobatidis* generally, and in particular in having certain hooks on the proboscides pointing anteriorly, and in the pore being situated posteriorly. It appears to differ from it, however, in the shape of the head generally, in the form of the bothridia, and in having a double row of hooks pointing

anteriorly. The worm has also a somewhat close resemblance to that of *R. curtum*, Linton, 1909, from which it only differs: (1) in being much larger, (2) in the relative size of the bothridia to the rest of the head, and (3) in the form and size of the posterior segments.

The armature of the proboscides in *R. curtum* has not been described.

These differences, however, do not appear to the author to be of much specific value and accordingly the worm described above, and also Linton's *R. curtum*, are considered identical with *T. rhynchobatidis*, Shipley and Hornell, 1906.

### *Rhynchobothrius longicollis* (van Ben., 1849) (fig. 10).

SYNONYM :—*Tetrarhynchus leucomelanus*, Shipley and Hornell, 1906.

One specimen of what appears to be this species was obtained by the author from *Trygon sephen*, Portugal Bay, November 7th, 1910; other specimens from *Rhynchobatus djeddensis*, February 3rd, 1911; *Trygon kuhli* and *Trygon walga*, November 27th, December 3rd, 1910, and February 7th and February 21st, 1911.

Shipley and Hornell gave the following diagnosis of this species :—

'Five centims. to eight centims. long, with posteriorly thick, stout proglottides, three millims. broad. Anterior half or two-thirds of the preserved body white, the remainder slaty black, deepening into a dense black. When alive, milky white, with a pink patch behind the proboscis sheath. Head with shallow lappets, well defined. Proboscides with an enormous number of very minute teeth, all uniform size and shape, arranged in rings and longitudinal rows. The proboscis sacs are very long, occupying seven-tenths of the length of the head. There is a short neck; the posterior edge of each proglottis is salient. Generative pores irregularly alternate. Habitat :—Intestine of *Trygon sephen*.'

The specimens agree closely with Shipley and Hornell's description of this species. The hooks on the proboscides are all alike and are extremely minute. As the anatomy of this species has not been described, the following notes are added :—The genital pores are irregularly alternate and are situated a little distance behind the middle of the lateral margin of the segment.

*Testes*. The testes vary in number from about 165 to 210; they occupy the entire dorsal area in front of the ovary. Aporally there are from about 90 to 110 testes; and porally there are from about 25 to 40 testes posterior to the cirrus pouch, and about 50 to 60 anterior to that organ.

*Vas deferens.* The cirrus pouch is small and almost globular, not measuring more than one-fifth the transverse axis of the segment. From the preparations it was impossible to decide whether the cirrus was armed or not. Median to the pouch the vas deferens forms a conspicuous coiled mass.

*Ovary.* This is a large bilobed organ situated posteriorly. Details relating to the vagina could not be made out in whole mounts.

*Vitelline glands.* These encircle the entire segment except for a small area dorsally and ventrally.

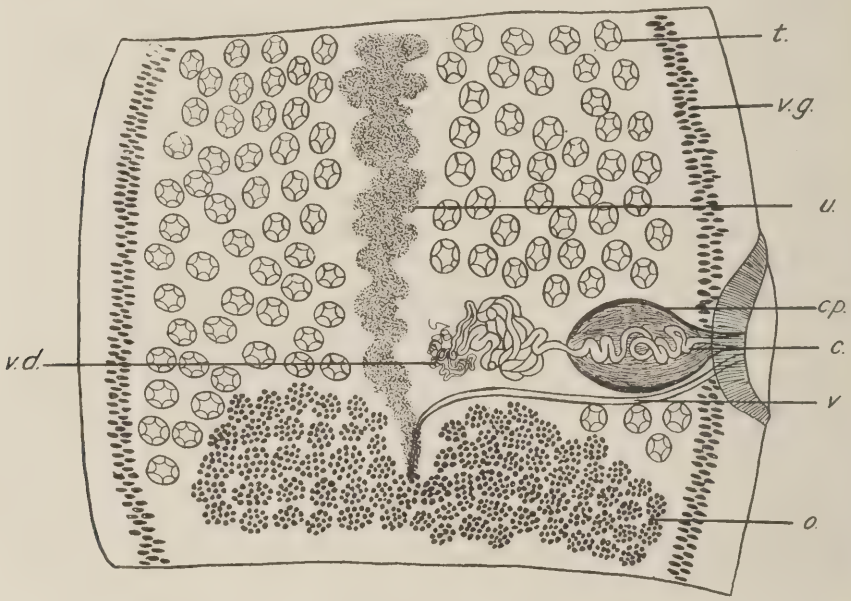



Fig. 10. *Rhynchobothrius longicollis.* Mature segment. *t.*—testes; *v.g.*—vitelline glands; *u.*—uterus; *c.p.*—cirrus pouch; *c.*—cirrus; *v.*—vagina; *o.*—ovary; *v.d.*—vas deferens. × 69.

*Uterus.* At first this consists of a coiled tube running in the median line from the ovary to the extreme anterior margin of the segment. When fully developed it consists of a bag with sacculated walls entirely filling the segment. The uterus was full of immature eggs.

Shipley and Hornell's species is indistinguishable from, and *T. ruficollis* (Eysenh., 1829) is closely related to, *Tetrarhynchus longicollis*, van Ben., 1849.

*Rhynchobothrius spinuliferus* (Southwell, 1911).

SYNONYMS :—*Tetrarhynchus spinulifera*, Southwell, 1911.
*Rhynchobothrium laciniatum*, Yoshida, 1917.

Two specimens were examined, taken from the intestine of *Rhynchobatus djeddensis*, Ceylon Pearl Banks, 1908.

EXTERNAL ANATOMY. The worm measures up to 5·5 cms. in length and the greatest breadth is 1 mm. It is composed of a large number of segments, the last measuring about 1·3 mm. in length. The posterior margins of the segments are produced into long digitate flaps with pointed extremities ; these laciniae are small in the neck region and short and blunt in gravid segments. The pores are situated laterally at the junction of the anterior two-thirds and posterior third of the segment. A uterine pore was present on the ventral surface ; the segments do not leave the chain until the uterus is fully mature. The neck is short, measuring only about 250$\mu$.

*Head.* The head is very small, measuring 1 mm. in length ; its breadth at the bulbs is 120$\mu$, and in the vicinity of the sheaths, 76$\mu$ ; the two bothridia measure 126$\mu$ in length and 90$\mu$ in breadth ; the proboscis sheaths form long, dense, spiral coils, and the proboscis sacs measure 280$\mu$ in length and 27$\mu$ in breadth. Unfortunately, the proboscides were not protruded and consequently details relating to the spines cannot be given. The entire head is covered with very minute spinules.

INTERNAL ANATOMY. Owing to the scarcity of material the muscular, excretory and nervous systems were not examined.

*Testes.* The number of testes could not be counted, as the worms were not well-preserved.

*Vas deferens.* The cirrus pouch is large, extending to the median longitudinal axis of the segment. The cirrus is dilated near the pore, and a number of coils of the vas deferens lie within the pouch. Outside the pouch the vas deferens forms a small coiled mass near the median extremity of the pouch.

*Ovary.* This is a bilobed organ situated posteriorly and comprised of a few, large, club-shaped acini. When fully mature the acini appear to fuse on each side, giving the ovary a dumb-bell appearance.

*Vagina.* Unfortunately, details relating to this organ could not be made out.

*Vitelline glands.* These encircle the entire segment and are composed of large acini.

*Uterus.* This develops early as a tube with very thick lobulated lateral walls ; it eventually fills the entire segment. A uterine pore is situated ventrally near the median extremity of the cirrus pouch ; it has a muscular margin.

*Eggs.* No fully ripe eggs were seen ; of those observed some were flask-shaped, with a number of short filaments at one end, whilst others were somewhat kidney-shaped, with a number of short filaments at both extremities.

There is no room for doubt that *R. laciniatum*, Yoshida, 1917, is identical with *R. spinuliferus* (Southwell, 1911).

### *Rhynchobothrius carcharidis*, Shipley and Hornell, 1906.

SYNONYM :—*Tetrarhynchus carcharidis*, Shipley and Hornell, 1906.

Two specimens of what appears to be this species were obtained from the intestine of *Trygon walga*, Ceylon Pearl Banks, 1910 ; collected by the author.

EXTERNAL ANATOMY. The worms measured up to 25 mm. in length and the greatest breadth was about 1 mm. ; the last segment, which was gravid, measured 1·2 mm. in length, and 1 mm. in breadth ; in all, about ninety segments were counted under magnification, but only the last ten or so were relatively large. The genital pores are irregularly alternate and are situated laterally in the posterior third of the segment.

*Head.* The length of the head cannot be given, as it passes imperceptibly into the neck. The length from the anterior extremity to the proboscis bulbs is 1·5 mm. and its breadth is 0·4 mm. The two bothridia measure 0·5 mm. in length and 0·37 in breadth ; their margins are not thickened. The proboscides are armed with a large number of extremely small uniform hooklets. The neck is short, but varies in length.

INTERNAL ANATOMY. The muscular, excretory and nervous systems were not investigated.

*Testes.* These are very numerous and lie on each side of the median longitudinal axis until they are mature, when they completely fill the central field.

*Vas deferens.* The cirrus pouch is pyriform and extends almost to the centre of the segment; within it the vas deferens lies in several coils; it was impossible to decide, in whole mounts, whether the cirrus was armed or not.

*Ovary.* This is a very massive, bilobed organ situated posteriorly, and composed of acini densely crowded together; the vagina could not be traced in whole mounts.

*Uterus.* This appears very early and at first consists of a tube running in the median longitudinal axis of the segment. Its lateral walls become lobulated, and eventually it fills the segment and becomes full of eggs; the latter were immature and, in whole mounts, details relating to them could not be ascertained.

The worm agrees with Shipley and Hornell's description of this species except that it is larger, contains more segments, and these are not so elongated as in the type species. In all probability, Shipley and Hornell's specimen was immature and had been preserved in an elongated condition.

### *Rhynchobothrius binuncus*, (Linton, 1909).

SYNONYM :—*Rhynchobothrium binuncum*, Linton, 1909.

One specimen of this worm was obtained by the author from the intestine of *Trygon* sp. (*walga ?*), Ceylon Pearl Banks, 27.11.10.

The species is distinguished by the peculiarly shaped hooks on the proboscides, by the worm being composed of about seven segments—the last being almost as large as the remainder of the worm,—and by the pore being situated in the posterior third of the segment.

### *Syndesmobothrium*, Diesing, 1863.

SYNONYM :—*Symbothrium*, Diesing, 1850.

This genus is characterised by Diesing as follows :—

*Body articulate, taeniaeform; neck tubular, rounded at the base; head tetragonal, with four terminal prominent bothria attached to head by posterior margin, cruciformly disposed, oval, slightly convex, joined with each other at the base by a membrane; proboscides four, filiform, armed, each one running through a bothrium (pedicel), excurrent at apex, long, retractile in the neck. Genital apertures marginal (?). In intestines of marine fishes of tropical America* (after Linton).

*Syndesmobothrium rubromaculatum* (Diesing, 1863) (fig. 11).

SYNONYMS :—*Tetrarhynchus rubromaculatus*, Diesing, 1863.
*Tetrarhynchus platycephalus*, Shipley and Hornell, 1906.

One specimen of what appears to be this species was collected by the author from the intestine of *Trygon kuhli*, Ceylon Pearl Banks, 1910. The proboscides were not protruded and it was therefore impossible to see the hooks properly. At least one longitudinal row appeared to point anteriorly. The worm is

FIG. 11. *Syndesmobothrium rubromaculatum*. A hook, from proboscis. × 500.

composed of about ten segments, the last one being greatly elongated. The genital pore is situated in the posterior third of the segment. As the only specimen available was in poor condition, the general anatomy of the worm could not be made out. The species is somewhat closely related to *Rhynchobothrium benedeni* (Crety, 1890).

*Otobothrium*, Linton, 1890.

*Body articulate, taeniaeform, head separated from body by a neck. Bothria two, opposite, lateral, each with two supplemental ciliated pits at the posterior free angles. Proboscides four, terminal, filiform, armed, retractile in neck. Reproductive apertures marginal* (Linton).

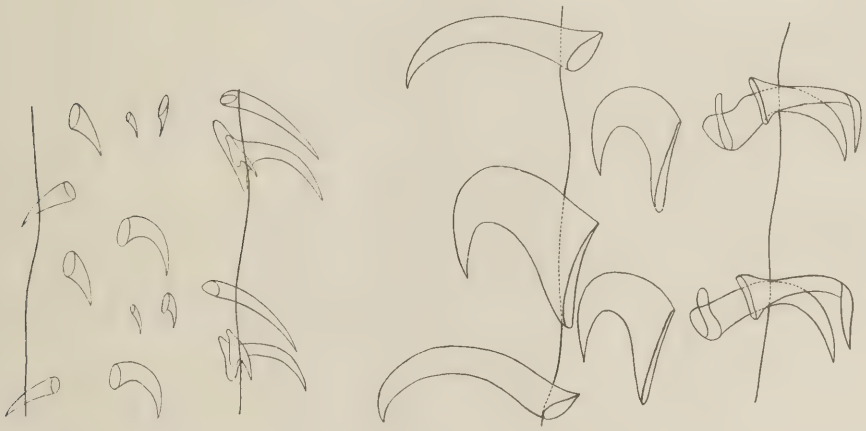
*Otobothrium magnum*, n.sp. (figs. 12-14).

Three specimens collected by the author from the intestine of *Rhynchobatus djeddensis*, Pearl Banks, Ceylon, 25.4.1909.

EXTERNAL ANATOMY. The worms measure about 35 mm. in length and have a maximum breadth of about 0·5 mm. ; there are about fifty segments, the last one measuring from 2 to 3 mm. in length. The lateral margins are straight and the posterior margins are not salient ; the genital pores are irregularly alternate and are

situated on the lateral margin, in the posterior third of the segment. The neck is very short, measuring only 60$\mu$ in length.

*Head.* The entire head has a length of 4·5 mm. and a maximum breadth of 1·3 mm. ; the two bothridia each have a length of 1 mm. ; the proboscis sacs have a length of 1·75 mm. and are marked by fine diagonal lines crossing each other at right angles. The proboscis sheaths are spiral, and those portions lying posterior to the bothridia have a length of 1·75 mm. The proboscides are practically as long as the entire head, and are armed with hooks of various sizes and shapes arranged in horizontal rings. The hooks towards the tip are smaller



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FIGS. 12 and 13. *Otobothrium magnum*, n.sp. Hooks from different parts of proboscis. × 250.

than the rest ; in each ring there are eight or nine hooks, the smallest hooks being situated in an antero-posterior line and the largest hooks on another antero-posterior line on the opposite side of the proboscis. Hooks of intermediate size and shape occur on the portions of the proboscides between these two lines.

INTERNAL ANATOMY. Owing to the material being scanty, the muscular, excretory and nervous systems were not investigated.

*Testes.* These first appear in about segment 32 and number about 300 ; their arrangement is peculiar in that about 50 testes lie posterior to the ovary and a separate cluster of about ten testes lie near the median extremity of the cirrus pouch. The remaining testes are distributed on each side of the middle line, in front of the ovary. The cirrus pouch was not fully developed and therefore

cannot be described; it appeared, however, to lie internal to the water vessels and to extend to the median antero-posterior axis.

*Ovary.* The ovary was rudimentary, bilobed, and situated a little distance from the posterior margin of the segment.

*Uterus.* This organ was also in a rudimentary condition and consisted of a slight irregular thickening running along the median antero-posterior axis of the segment.

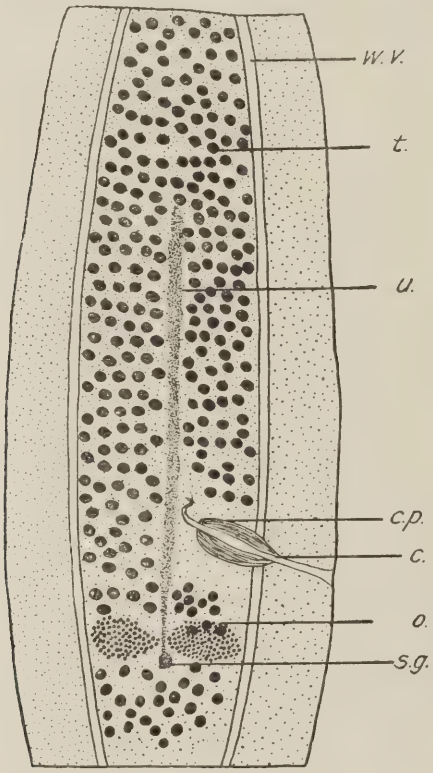


FIG. 14. *Otobothrium magnum*, n.sp. Mature segment. *w.v.*—water vessel; *t.*—testes; *u.*—uterus; *c.p.*—cirrus pouch; *c.*—cirrus; *o.*—ovary; *s.g.*—shell gland. × 112.

DIAGNOSIS. There are only four species belonging to this genus and they have all been described by Linton, viz.,

*O. crenacolle*, Linton, 1890 (described from an adult worm);

*O. dipsacum*, Linton, 1897 (described from a larva);

*O. insigne*, Linton, 1905 (described from a specimen not fully adult) ;

*O. penetrans*, Linton, 1907 (described from a larva).

*O. crenacolle* measures about 14 mm. in length, is composed of about twelve segments ; the genital pores are situated at the middle of the lateral margin of the segment.

*O. insigne.* In this species the genital pore is situated in front of the middle of the lateral margin of the segment ; the head terminates posteriorly in a ' collar ' which surrounds the anterior part of the strobila. The worm was not fully mature ; it measured 10 mm. in length and was made up of twelve segments.

*O. penetrans.* In this species the chief characteristic appears to be the fact that, posteriorly, the proboscis sacs are divergent.

*O. dipsacum.* The chief characteristics of this species (which, as noted above, was described from a larva) is the fact that each proboscis

' Has a longitudinal line towards which the short diagonal rows of hooks converge on each side. Near the base of the proboscides, where the hooks are somewhat scattered, from six to ten hooks to a row could be counted on each sac of the horizontal line under favourable circumstances. More than twice that number could be counted in the rows nearer the apex of the proboscis.'

*O. magnum*, n.sp., resembles *O. dipsacum* in having an antero-posterior line on each proboscis towards which the hooks gradually get smaller, but the number of rows of hooks is much less than that given for *O. dipsacum* and they differ also in shape. For these reasons the species is separated from all the species of *Otobothrium* hitherto described. It further differs from *O. crenacolle* and *O. insigne* in size and in the position of the genital pore. Type species are in the Liverpool School of Tropical Medicine.

### *Otobothrium dipsacum*, Linton, 1897.

Several large larvae from the mesenteries of *Serranus undulosus*, South Silavaturia, Ceylon Pearl Banks. Collected by the author, April 2nd, 1909 ; also from *Diagramma crassispinum*, and *Balistes* spp., Ceylon Pearl Banks.

### Genus *Coenomorphus*, Loennberg, 1889, emended.

*With the characters of the family.*

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# PREFERENTIAL AND COMPULSORY BREEDING PLACES OF *AEDES (STEGOMYIA) AEGYPTI* AND THEIR LIMITS

BY

H. R. CARTER

ASSISTANT SURGEON-GENERAL U.S. PUBLIC HEALTH SERVICE

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Of course, the one absolute material requisite, material as distinguished from conditional requisites as temperature, etc., for the breeding of mosquitos of all kinds and in every place is *water.* Fruitful oviposition of this insect then in nature takes place only in relation to water; present at the time of oviposition for such as deposit their eggs only in water; or water in the future for such as deposit their eggs in places free from water, but which will be covered by it at the proper time for them to hatch.

Given water then, in *esse* or in *posse,* it is common knowledge that some species, even some genera, of mosquitos show decided choice in the nature of the places they select for oviposition and, where such places are available, use them to the exclusion of other collections of water, or at least so much more frequently that other places are not a serious factor in the propagation of this mosquito. Nevertheless, we sometimes find that when breeding-places of the preferred kind are *not* available, this mosquito will deposit eggs in other collections of water, although it may be not in all classes of such collections.

The class of breeding-places first mentioned—*i.e.,* those utilized for oviposition when all kinds are available, we may call the *preferential breeding-places,* or the *breeding-places of election,* for the mosquito in question. The second kind—*i.e.,* those in which

eggs are deposited when, and only when, breeding-places of election are not available, may be as properly called *compulsory breeding-places* and naturally the places in which, in nature, eggs are not deposited even when they are the only collections of water available, are beyond the natural limits of even compulsory breeding-places and from the last two we may be able to determine the limits of breeding for this mosquito in nature.

Note here that when we speak of oviposition or of breeding in any place or class of place, we mean oviposition or breeding sufficient to be a serious factor in the propagation of this mosquito. When less than this it is considered negative. This is, of course, rather the view-point of the sanitarian than that of the entomologist, yet for the sanitarian it is quite consistent. In *Anopheles* surveys we grade breeding-places as 'of sanitary importance' and 'of no sanitary importance,' and disregard the latter. In yellow fever campaigns we count our work complete when the production of *Stegomyia* is so reduced that yellow fever cannot be kept up by them, although a minimal breeding may still be going on. So the occasional finding of a few larvae of a certain mosquito in places usually free from them does not justify our putting such places in the same class with those in which larvae are habitually found in numbers. In sanitary matters, and I confess that I am inclined to look at mosquitos from a sanitary standpoint, no one cares about *a* mosquito. It is only when zeros accumulate behind them and they show as hundreds, thousands, or ten thousands that they become important—to the sanitarian, I mean.

What we have said of the relation of oviposition to the different classes of possible breeding-places may be true of all kinds of mosquitos ; it is true of many kinds, but I am confining myself here and now to one : *Aedes* (*Stegomyia*) *aegypti**—the known vector of yellow fever and dengue. I had purposed to take up the same question about some species of *Anopheles*, malaria vectors, but this would have made this article entirely too long,—a book not a paper.

For this mosquito, it is a matter of common knowledge that its breeding-places of election are collections of water in artificial (man-made) containers, or things that simulate them : wood, cement, stone and brick being preferred to metals as the material for such

* = *Stegomyia fasciata*, Fabr. Editors.

containers and wood preferred to cement. Calabashes have seemed to be especially attractive to them. Yet long ago my attention was called to the fact that these conditions of breeding, although usual, were not obligatory and, if lacking, could be substituted by others. I was in Panama City in the last part of November or very early in December of 1905, and was there inspecting the *Stegomyia* work. The man immediately in charge was a very capable one, and we visited together a number of premises, houses and patios. In all, the *depositos* of water kept for domestic use were protected against access of mosquitos. Cisterns, *tinajas* and *ollas*, all were either empty or covered, some with wire-netting, some with cloth tied on and some with well-fitting, solid covers; no water in any of them was accessible to mosquitos. Of a considerable number of premises he had inspected before I came, he reported all as being negative for larvae and in none that I examined with him could I find larvae or pupa-casts floating on the surface ; in large collections of water the latter show up far more readily than larvae themselves.

Yet in one place I was able to show him *Stegomyia* larvae, possibly ten or a dozen, and maybe twice as many, taken with a tea-spoon from a rot-hole in a tree, small ones, in the little collections of water in the axils of the leaves of some ornamental plants in the patio—a species of Colocasia. Guessing the cause for the oviposition in these places, I directed him to place, and assisted him in placing, a number of calabashes, *ollas*, and other containers of water in shady and partly dark places about the house and patio, with instructions to empty them every Saturday at the weekly inspection. The result was successful: not only was this patio free from larvae for the next three or four Saturdays when I inspected it, but I am persuaded that neither this inspector nor any of his men ever failed to examine and to provide for the elimination from any other patio of all such breeding-places as those in which we had found these larvae.

As we had found practically all of the *depositos* of water—the preferential breeding-places of this mosquito—inaccessible to it in the vicinity in which we had found the larvae just mentioned, and had not found larvae in rot-holes in trees or the axils of Colocasia leaves elsewhere, it is fair to believe that the places in which larvae were found in this patio were breeding-places of compulsion, used

only because none more suitable were available and this seemed to be, to an extent, confirmed by the result of making suitable breeding-places accessible.

It may be noted here that while we had already occasionally found *Stegomyia* larvae in the small collections of water in the axils of the ornamental Colocasias, yet we had come to regard them as of minor sanitary importance, as so small a proportion of them developed into imagos. This, indeed, seemed to be generally true of the compulsory breeding-places of this mosquito. Her progeny seemed to have a much better chance of reaching maturity in the breeding-places of election than in those she utilized only when these were not to be had. This may not be without exception, but the one exception which I have seen reported involves an artificial condition and is not pertinent to our subject.

What are the limits to the compulsory breeding-places of this mosquito ? On the Isthmus of Panama we did not find its larvae in marshes nor in seepage out-crops—so favourite a place for *Anopheles*—nor in sluggish streams and never in a street gutter, nor a roadside puddle, except when we were reasonably sure that they had been washed in from some other place, as from an over-flowing cistern or a sagging house-gutter, and this was true whether more suitable breeding-places were available or not. In other words, such places were beyond the natural limits of breeding—complete breeding, from oviposition to imago—of this mosquito on the Isthmus of Panama.

Is this true everywhere, or, as happens sometimes with other mosquitos, is there a regional variant to this part of its biology ? Some years ago Francis, of the U.S. Public Health Service, as a result of his observations for some three years in and about Mobile, Ala., stated that ' larvae of this mosquito have not been found in any collection of water the bottom of which was of mud.' As intended, ' not in mud-puddles,' this seems correct and would be so accepted, I think, by all of you. As expressed, it doubtless was correct, anyway. The implication, however, that this was a general rule and that the mud bottom of a collection of water was the factor which determined that *Stegomyia* should not breed therein is erroneous. During 1920 and 1921 in Payta, Sullana, Piura, Catacaos, and in practically every other town in Northern Peru

which we examined, we found *Stegomyia* breeding and breeding freely, in the great *botijas*, or *tinajones*, used to store water in that country, although their bottoms were generally covered with mud, from six to ten inches deep. Very evidently, mud at the bottom of the water in no wise prevented their breeding in it.

Admitting that we have not found them breeding completely in mud-puddles, what are the factors which prevent this ? I think we can name one of them, and possibly the determining one. At Catacaos, a town of about 40,000 people in Northern Peru, where we had yellow fever in 1919 and 1920, there were many wells, say from 100 to 200. They were merely holes dug in the ground from six to twelve feet deep according to the depth of the ground water. We never found *Stegomyia* larvae in these wells, even when breeding was fairly well-controlled in the artificial containers in their neighbourhoods. Plenty of larvae of other mosquitos, but none of *Stegomyia*. At Casa Grande, also in Northern Peru but further south, in every house of a skilled employee there was a well and we found *Stegomyia* larvae in exactly 100 per cent. of these wells ! Why this difference ? Could it be because the wells at Casa Grande were in houses occupied by men while those at Catacaos were in the open ? It did not seem so, because the wells for general service at Casa Grande were out of doors and all that we examined, some eight or ten, showed *Stegomyia* larvae, although fewer than those in the houses. There was another difference between the wells at the two places besides their locations in, and not in, houses. Those at Casa Grande were lined with brick and cement, while those at Catacaos were unlined, simply dug in the clay. In Chiclayo, too, situated between the two places, there were a number of wells, some lined and some unlined. In none of those unlined which we examined did we find *Stegomyia* larvae ; in none of those lined, whether with wood or with brick and cement, did we fail to find them !

Really this explanation had been anticipated. Anyone who has seen the oviposition of this mosquito standing just at the edge of the water, sometimes on the water, sometimes on the container, sometimes partly on both water and container, would naturally guess that the physical nature of the sides of the container *at the water's edge* might well be a factor in her choice of a place for oviposition ; while, unless it affected the quality of the whole body of water, the nature of the bottom would be unknown to her.

After considerably more observation on this point in Peru by Hanson and by Dunn and their men and much inquiry, verbal and by letter, of men doing *Stegomyia* control work in other countries: Mexico, Yucatan, Central America, Panama and Colombia, I felt myself justified in presenting this formula, modelled on that of Francis, as limiting the breeding-places of this mosquito:

'*We have not found this mosquito* (*Aedes* (*Stegomyia*) *aegypti*) *breeding, in nature, completely, that is, from oviposition to imago, in any collection of water, all the sides of which at the water's edge were of mud.*'

It was thus given out in a lecture to the Laboratory Class of Officers of the U.S. Public Health Service in Washington in the Spring of 1923, and, a little later, to the class at the School of Hygiene and Public Health of The Johns Hopkins University in Baltimore. As expressed it was correct; neither myself nor anyone of whom I had inquired had ever seen *Stegomyia* breeding in the places excluded by this formula. Even the implication was logical and I then thought correct, yet I have recently learned that, taken as a universal formula, it is not.

Last September I received, through the International Health Board of the Rockefeller Foundation, copies of official reports sent them from the British Colonial Office of the yellow fever epidemics of 1922 and 1923 in British West Africa. Among these epidemics was one at Salt Pond on the Gold Coast, small but virulent, as African yellow fever has usually proved to be. In the report on this it was stated that the principal source of *Stegomyia aegypti* at this place was the lagoon and the tracks in the mud around it of the men and boys who go bathing there. This production was stated to be large and to be uncontrollable except by a major engineering project. As illustrating the amount of this breeding, the reporter stated that in one collection of water (in this mud) of about ten inches square, characterized by him as 'a typical *Anopheles* breeding-place,' the larvae were so abundant that 'they nearly filled the saucer in which they were dipped up' and it was added that these larvae developed into imagos of *Stegomyia aegypti* and *Culex fatigans*.

The writer of this report, Dr. Lorena, and his immediate superior, Dr. Watt, both officials of the Sanitary Service of the Colony, ascribed this breeding 'in such unusual places' to the complete

elimination of the usual breeding-places from the town and its environment.* This, of course, would make the breeding of *Stegomyia aegypti* in and about the lagoon a compulsory breeding, there being no more suitable place available. Yet there are other reports, previous to the one quoted, implicitly accusing the Salt Pond Lagoon and its environment of producing *Stegomyia aegypti* normally—*i.e.*, when there had been no interference with their normal breeding-places. That of Drs. Horn and Tytler (Oct. 16, 1920), made in connection with the Yellow Fever Commission of the International Health Board of the Rockefeller Foundation of 1920—the one on which Gorgas was serving when he died—implies the production of *Stegomyia* in and about this lagoon, as it suggests the canalization of this lagoon as affecting the prevalence of yellow fever in Salt Pond. It also states that the same conditions exist at Seccondee and other places on this coast as at Salt Pond. In some of the reports, too, of the British West African Yellow Fever Commission—the last I saw were, I think, of 1913—and the papers transmitted with them, there were also recommendations for the abolition of this lagoon and cure of the mosquito breeding conditions around it, on account of their effect on the propagation of yellow fever at Salt Pond.

Be this as it may, neither Le Prince nor myself nor any one of the many whom I have consulted have ever seen, in the Americas, *Stegomyia* breeding, from oviposition to imagos, in mud-puddles. Nor have either of us even seen this mosquito breeding anywhere in anything like the profusion implied in this report. *Culex fatigans*, yes; *Stegomyia*, never. It is a pity the reporter did not give the proportion of the two kinds of imagos developed from his pool in the mud.

Nevertheless we cannot, on this account, reject this report as erroneous. I have seen too much of what, I suppose, may be called 'regional variation in the biology of mosquitos' to refuse credence to a well-attested statement about them, as I consider this to be, simply because it is not in accordance with my own observations made in an entirely different region. And, while I would greatly like to examine for myself the breeding of this mosquito in and

* Why, knowing this, the 'usual breeding-places' in the town and its vicinity were not at once re-established one cannot imagine. Knowing where they were these could have been easily controlled; the breeding about the lagoon had been reported as 'uncontrollable.'

about this lagoon at Salt Pond, yet I feel that we must accept the statements of fact as given in the report quoted. Without denying it I am, however, less inclined to accept the breeding of *Stegomyia aegypti* in and about the lagoon at Salt Pond *as its normal breeding there—i.e.*, as taking place when water in artificial containers was available to them for oviposition, because (1) the evidence for it that I have seen is implied rather than direct and is less in detail, (2) it seems to me antecedently more improbable, and (3) the characterization of these places, the lagoon and the tracks around it, in Lorena's report, as 'unusual breeding-places' for this mosquito, used only because the usual ones in the neighbourhood had been eliminated.*

In any case, however, we must change the formula just given you by limiting it to the Americas. And I am entirely willing to do this because all that I know of *Stegomyia* breeding at first-hand is its breeding in the Americas. Our formula should then read:

'*In the Americas* we have not found this mosquito, *Aedes* (*Stegomyia*) *aegypti*, breeding, in nature, completely, from oviposition to imago, in any collection of water all the sides of which at the water's edge were of mud.'

This is not only correct as expressed, but its implication that collections of water in the Americas under the conditions specified are not found breeding *Stegomyia* in nature is, I am inclined to think, correct also. For, since the receipt of the West African reports mentioned, I have been in communication, in reference to the breeding of *Stegomyia aegypti* in puddles, with a number of men whom I know and who are working, or have been working, in the Americas, for the elimination of yellow fever: with White, Scannell, Walcott and others in Brazil; with Hanson and Dunn, an entomologist, in Colombia; with Caldwell, Connor, Scannell and Houle in Mexico and Yucatan and with Connor in the Guianas, and no one has, so far, reported having found *Stegomyia* breeding in nature completely in mud-puddles and a number report directly to the contrary. I think then that, for the present at

* After the reading of this paper the writer had opportunity to talk to Dr. A. E. Horn, to whose report of 1920, made jointly by himself and Dr. Tytler, allusion has been made. Dr. Horn stated that he had not seen larvae of *Stegomyia aegypti* in the Lagoon at Salt Pond, nor in the puddles adjacent thereto, nor had he seen them in similar places at Seccondee. The inference then drawn from the Report of Drs. Horn and Tytler, although logical, was incorrect.

least, the implication of our modified formula, that complete breeding of *Stegomyia* in mud-puddles does not, in nature, occur in the Americas may be accepted. It is supported by a considerable amount of evidence; negative evidence, indeed, but from the nature of the question no other kind is here possible. Is it enough—negative evidence is convincing in proportion to its mass—to justify us in asserting that, in the Americas, mud-puddles are beyond the limits of natural *Stegomyia* breeding—even of natural compulsory breeding? I think so, but we should know when the work in Brazil is finished.

At any rate, there seems to be a difference in the limits of the natural compulsory breeding-places of this mosquito in the Americas and at Salt Pond and, it may be, in other places in West Africa. Possibly the biology of the African strain is less rigid than is that of ours; that it can adapt itself to conditions which the American strain is unable to meet. Well was it for us that this last was true! Had the Salada and all the puddles about Guayaquil produced *Stegomyia*, the elimination of yellow fever from that city had been more difficult even than it proved. So if the conditions of breeding as reported at Salt Pond are general in West Africa, the elimination of yellow fever therefrom will be decidedly more difficult than if the limits of the breeding of this mosquito, both normal and compulsory, were there the same as we have found them to be in the Americas.

Very obviously, then, the utilization by *Stegomyia aegypti* of breeding-places other than those of election is of decided importance to the sanitarian. It makes its control more difficult than if only the latter were used. If, as at Salt Pond, one must control its production not only from artificial containers in the town, but from mud-holes and ponds as well, the difficulty may be many-fold greater than if the first only were involved. And this difficulty might be *very* greatly increased if this adaptability of the insect to breeding in what are now abnormal places should prove to be a characteristic which can be cultivated and increased by practice, as has apparently happened with at least one characteristic of *Anopheles maculipennis*.*

It would seem advisable then so to plan our methods of *Stegomyia* control, as to limit as much as possible the use of compulsory breeding-places by this mosquito. This depends on the facilities

* The development of a preference for the blood of domestic animals over that of man in these mosquitos in certain parts of France and Denmark.

available for oviposition. As long as the mosquito has easy access to a sufficiency of breeding-places of election, she will not seek to deposit eggs in any other. *It may be advisable then to provide her with such breeding-places of election.*

Does to provide suitable breeding-places for, and easy of access to, this mosquito, of which we are trying to rid ourselves, seem a paradox? I have been asked (about *Anopheles*, however) if I 'wished to encourage them to breed?' Not exactly, but we don't care whether they breed or not, *provided there be no production.* Eggs, larvae and pupae are absolutely innocent, and if breeding is stopped short of their final development into the imago, which alone is offensive, we are satisfied. Eggs and larvae then, as many as you wish (pupae are too close to the final change to risk) provided there is no development of imagos.

Control of this mosquito then* by measures which allow of oviposition in their preferential breeding-places may be, in certain places will be, the preferential method to adopt.

Now by the use of fish, by oiling (not usually a method of election), and by emptying and refilling water containers at proper intervals of time, we can, in general, sufficiently control the production of *Stegomyia* and yet allow them access to their preferential breeding-places for oviposition. These methods then involve no risk of driving them to unusual breeding-places, the control of which may be difficult. Nay, even the removal of containers and the covering them so as to exclude access of mosquitos, methods very advisable for permanent work, can be used if other suitable breeding-places be provided accessible to them. The production of imagos from these breeding-places provided can be controlled, sometimes by fish or, as we know where we placed them, easily and absolutely by emptying them at the weekly inspection

It may be worth mentioning to you that a small amount of sugar added to the water is reported by Fielding to increase its attraction to this mosquito for oviposition, although her progeny do not thrive on it: an instance, and they are rare among the lower forms of life, in which the maternal instinct is at fault.

Very obviously, then, before systematic work against yellow fever is begun in West Africa a survey must be made to determine the

* This is a general rule applying to *Anopheles* no less than to *Stegomyia aegypti.*

limits there of both the normal and the compulsory breeding-places of its yellow fever vector, or vectors. If the compulsory breeding-places be beyond our ready control, we should, by some of the methods just given, try to arrange for the control of normal breeding, so as to produce little or no compulsory breeding, remembering always that less than a 100 per cent. reduction of *Stegomyia* will eliminate yellow fever.

If the normal breeding habits of this mosquito in West Africa generally are those implied by the report of Horn and Tytler and similar reports as obtaining at Salt Pond and Seccondee,* the elimination of yellow fever from that region would be, I think, impracticable by the methods we have used in the Americas.†

* Sir Rubert Boyce also notes having seen *Stegomyia* breeding in a pool on the mud-covered roof of a hut (*i.e.* 'puddle breeding') in West Africa. I think he does not state where.

† See footnote: page 500.

# TWO NEW SPECIES OF REPTILIAN CESTODES

BY

PROFESSOR O. FUHRMANN, PH.D.
(NEUCHÂTEL, SWITZERLAND).

(*Received for publication* 9 *October*, 1924)

[These two new species were sent to us for determination by Dr. H. O. Mönnig, Research Officer, Ondersteppoort, Pretoria, to whom we here tender our thanks.]

### *OOCHORISTICA THEILERI* n.sp.

Host: *Agama hispida?* Locality: Pretoria.

There are altogether forty specimens of this interesting worm, collected from a Lizard, which Dr. Mönnig believes to be *Agama hispida*, var. *distans*. With the exception of a single specimen to which we will refer later, all the mature specimens, *i.e.* specimens possessing egg-capsules containing embryos, are 4-8 mm. in length, with a maximum width of 0·85 mm. The scolex measures 0·3-0·4 mm. across the suckers, the latter measuring 0·14 mm. in diameter. The scolex is not separated from the strobila by any stricture or neck-like structure, and the latter maintains a uniform width of 0·76 to 0·85 mm. throughout its entire length. The segments are indistinctly separated from one another, and are all, excepting the last ones, wider than long.

The *Musculature* of the strobila is of special interest. Whereas the *Oochoristica* spp. usually possess two or three layers of longitudinal muscles, our species only possesses a single layer of bundles made up of longitudinal muscle fibres. A similar disposition is to be found in *O. zonuri* Baylis, only with this difference: that the latter species possesses 40 dorsal and ventral bundles made up of 15 to 20 fibres each, whereas *O. Theileri* possesses the weakest musculature that we have ever seen. There are about 25 bundles made up of 2 to 4, exceptionally of 5 to 6 longitudinal fibres. As these bundles are spindle-shaped, it often happens that on transverse sections there are only to be seen a few irregular bundles, between which

appear isolated longitudinal fibres. The transverse muscles are weakly developed, and the dorso-ventral ones very fine. Calcareous corpuscles are scarce.

The *Excretory System.* This presents the typical disposition of four winding vessels of which the two ventral ones are united by a fine network of secondary vessels. We have also noticed other ramifications, but have been unable to follow them up, owing to their minuteness.

The *Genital Organs* appear close behind the scolex, and develop very uniformly in all the specimens that we have examined. The male and female gonads attain their sexual maturity in the fifteenth to the eighteenth segment. They first of all develop slowly, then suddenly from the twelfth to the fourteenth segment onward they develop very rapidly. In the sixteenth to the nineteenth segment the ovary has almost entirely disappeared, and the egg-capsules have begun to form ; the formation of the latter is terminated in the next five to six segments, then follow a small number of gravid segments (see fig. 1 B). The strobila are thus constituted according to their length, of 23 to 28 segments. The genital pores alternate irregularly, and open into a remarkably deep genital atrium, situated slightly anterior to the middle of the border of the segment. The genital atrium (see fig. 1) is as large if not larger than the cirrus pouch, and presents two peculiarities, namely, it is lined with a cuticula armed with minute spines, and beneath which there is to be found a distinct layer of sub-cuticular cells from which arise a radiating musculature which is lost in the parenchyma.

The bottle-shaped cirrus pouch is 0·12 mm. long, and has a very muscular neck, although the distal extremity possesses but a weak muscular wall. When evaginated, the stout *cirrus* is 0·1 mm. long and measures at its base 0·04 mm. in diameter. The basal portion of the cirrus is covered with the same spines as those lining the genital atrium. The cirrus and *ductus ejaculatorius* are surrounded by a sheath of muscles. There is a well-developed retractor muscle attached to the cirrus pouch. The *vas deferens* presents a few loose coils. The *testes*, 26 to 30 in number, are entirely situated behind the female gonads, and form two groups, which may occasionally meet in the mid-line when the segments are much extended. This disposition has as yet never been recorded from *Oochoristica* spp.

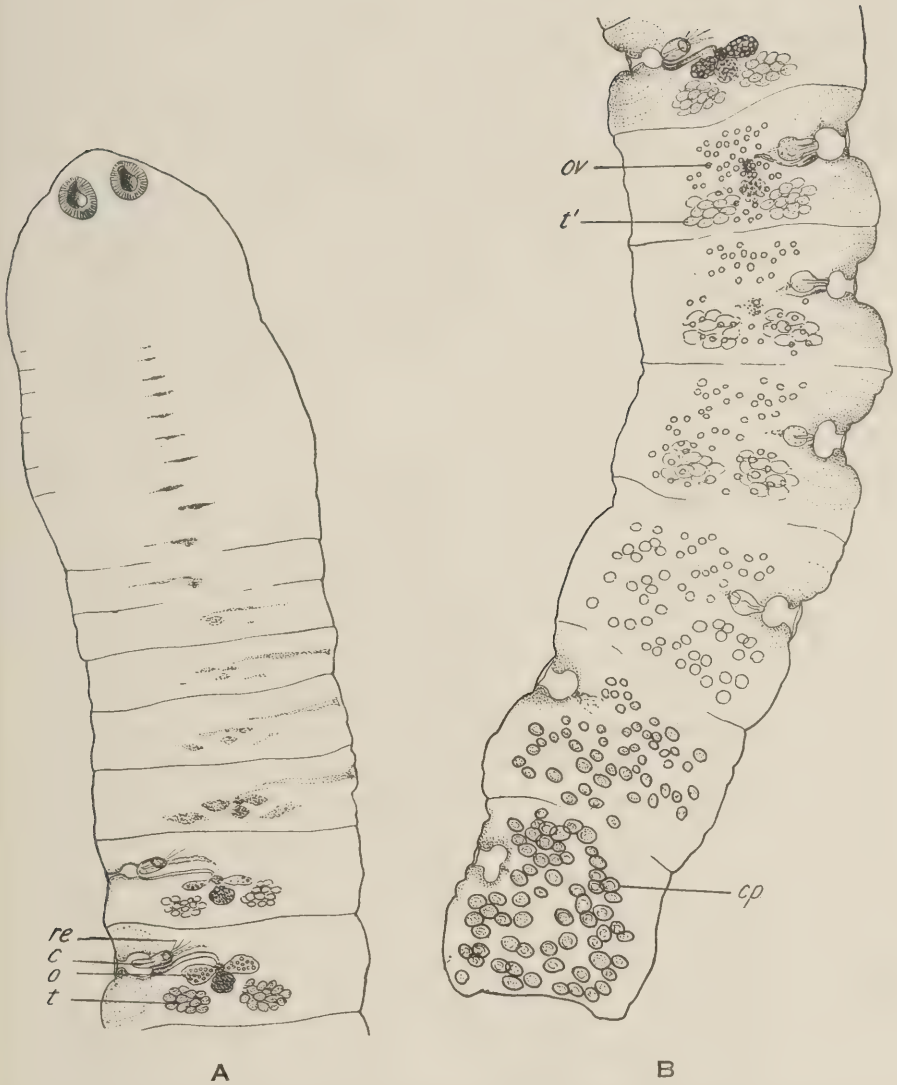


FIG. 1 A and B. *Oocboristica Theileri* n.sp. Mount of an entire worm. *c.*—cirrus; *cp.*—egg-capsules; *o.*—ovary; *ov.*—ova dispersed in the parenchyma; *re.*—retractor muscle of the cirrus pouch; *t.*—testes; *t'.*—testes in regression.

As is usually the case in this genus, the *vagina* opens behind and on the same level as the cirrus pouch, and presents in its proximal portion a region as long as the cirrus pouch, possessing a thick muscular wall, and of the same diameter as the cirrus ; the vagina then forms a narrow tube which dilates near the ovary to form a small *receptaculum seminis*. The *ovary* is slightly displaced towards the poral side of the segment, and attains a maximum width of 0·2 to 0·24 mm. It consists of two wings, oval in shape, and not lobed ; the poral wing is slightly smaller than the aporal one. The yolk gland is almost spherical, and measures 0·08 to 0·1 mm. in diameter. The shell gland is situated dorsally, and is 0·06 mm. in diameter ; it surrounds a very distinct ootype.

We have already remarked on the fact that both the male and female genitalia attain their sexual maturity in the same segment ; this is, nevertheless, contrary to what occurs in most Cestodes. The testes, however, still persist in several segments, whereas the ovary and the yolk gland disappear suddenly from one segment to the next. In mature segments, there appears between the male and female genitalia a sac-like uterus, which in the next segment has formed out-pocketings, especially anteriorly. These diverticula are filled with ova ; in the next segment, however, they form as many capsules as there are ova, each ovum being thus enclosed within a capsule. The segmentation which begins as soon as the ova enter the uterus, continues its course, and four segments further on, *i.e.* six segments posterior to the mature female gonads, the egg-capsules are definitely formed. These capsules can be easily distinguished, as they stain very deeply ; they seem to be formed of a hard and fragile substance, because on sections the capsules are broken into polygonal pieces showing a clean fracture. First situated in the field between the excretory vessels, the capsules partly push their way beyond the latter, and even beyond the nerve stems. The capsules are 0·06 mm. in diameter, and the embryos, surrounded by a very thin membrane, measure 0·03 to 0·04 mm. in diameter.

Whereas the number of ova contained within the ovary exceeds 200, we have never found more than 100 egg-capsules, each containing a single egg. One must, therefore, admit that when the ovary disappears suddenly, more than half the ova are unfertilized, and are resorbed. There are from 60 to 100 capsules per segment. On

several occasions we have found in a single strobila segments containing only 20 capsules, along with about 70 very small capsules presenting the aspect of calcareous corpuscles, and which are nothing else than aborted embryos, the abortion being due to inadequate fertilization.

As we have already mentioned above, our material contains a much larger specimen possessing the same anatomy. This species, *O. Theileri forma major*, presents, however, certain peculiarities with regard to the development of the sexual organs, and to the number of uterine capsules.

The specimen is 30 mm. long and has a uniform width of 1·7 mm. The *scolex* measures 0·5 mm. across the suckers ; the latter are small and only measure 0·14 mm. in diameter. Immediately behind the suckers, the scolex widens out to attain the width of the anterior part of the strobila.

The *Sexual Organs*, and especially the female gonads, attain their maturity only in the forty-fifth segment, whereas in the other species examined they were already mature in the sixteenth or seventeenth segment. In the forty-sixth segment the female gonads have entirely disappeared, and the ova are surrounded by the uterine capsules, which attain their full development in the seventieth segment. The last thirty-one segments all contain fully-developed capsules. With regard to the number of capsules, we find it to be twice as great as that for the small species ; this may perhaps be explained by the fact that the segments are twice as long as those of the other species.

There is no doubt that these two forms belong to the same species, and such differences undoubtedly arise from the fact that when the young Cestode arrives in the intestine of its host, it immediately developes its genital organs and ova ; later on this development slows, and the development of the genitalia and of the ova takes place much more gradually, *i.e.* occupies a much larger number of segments. Probably both these forms belong to two different infections.

Of the twenty-six species of *Oochoristica* known, seven are found in Africa, four occurring in Reptiles; these are: *O. zonuri*, Baylis, *O. agamae*, Baylis, *O. truncata* (Krabbe), and *O. crassiceps*, Baylis, all different from *O. Theileri* n.sp.

### *OPHIOTAENIA MÖNNIGI* n.sp.

Host : *Leptodira hotambeia.* Locality : Pretoria.

This typical Proteocephalid was collected from the intestine of a red-lipped Snake *Leptodira hotambeia.*

The scolex of this only specimen is missing, but the anatomy is sufficiently characteristic to create a new species, for which we propose the above name. This small worm probably does not exceed 50 mm. in length, the greatest width being 1·8 mm. The youngest proglottids are 0·09 mm. long and 0·78 mm. wide, the next segments, showing the genital primordalia, are 0·17 mm. long and 0·9 mm. wide, and the segments in which the genital organs have attained their full development measure 0·79 mm. in length and 0·9 mm. in width. The gravid segments, on the other hand, measure 2·5 mm. in length and 1·5 to 1·8 mm. in width. The segmentation of the strobila is indistinct, as is the case for all *Ophiotaenia* spp.

The *Muscular System* is chiefly formed of longitudinal muscles, whereas transverse and dorso-ventral fibres are scarce. In young proglottids in which the genital primordalia have not yet appeared, the longitudinal muscles form a circular layer 0·04 mm. wide, surrounding the medullary parenchyma : the latter is only 0·03 mm. wide and contains numerous transverse fibres, dorso-ventral fibres being very scarce. We have found occasional calcareous corpuscles. In gravid segments the longitudinal muscle layer is displaced towards the periphery of the segment because the medullary parenchyma is almost entirely occupied by the uterus, which increases the width of the latter by nearly five times. In these segments the longitudinal muscle fibres form a narrower and more irregular layer. Transverse and dorso-ventral fibres are exceedingly scarce.

*Excretory System.*—Two pairs of longitudinal excretory vessels are to be found throughout the entire strobila. The ventral vessels are thin-walled, and measure from 0·03 to 0·04 mm. in diameter. We have been unable to find commissurae nor have we seen any ramifications ; the latter must be scarce, contrary to what is found in the other species of *Ophiotaenia.* The dorsal vessels, 0·008 to 0·012 mm. in diameter, are to be found dorsal to the ventral vessels in the anterior part of the anterior region of the strobila, whereas in the posterior region the former are displaced towards the exterior (see

fig. 2 A). The walls of the dorsal are thicker than those of the ventral vessels and are surrounded by a layer of plasma containing occasional nuclei. In the anterior region of the strobila these vessels are surrounded by a single layer of stout longitudinal muscle fibres, the latter being derived from the longitudinal musculature. We have been unable to study the *foramina secunda* for want of material.

*Nervous System.*—The two longitudinal nerve stems, 0·03 mm. in diameter, are situated close to the lateral borders of the segment, and in places touch the sub-cuticular cell layer. The two accessory nerves, the one dorsal and the other ventral to the main nerve, are exceptionally distinct in the anterior part of the strobila.

The *Genitalia* develop slowly, and open into a fairly deep genital atrium, which alternates irregularly, and which is situated about the middle or slightly posterior to the middle of the border of the segment.

The male genitalia consist of a relatively small egg-shaped *cirrus pouch* with weak muscular walls. The distal portion of the pouch, contrary to what occurs in most of the *Ophiotaenia* spp., does not reach as far as the ventral excretory vessel. The total length of the pouch is 0·2 mm., and the diameter is 0·1 mm. It contains a large muscular cirrus. The coils of the *vas deferens*, situated outside the cirrus pouch and between the latter and the median stem of the uterus, are in places greatly dilated and can attain a diameter even greater than that of the cirrus pouch (0·1 to 0·12 mm.). This portion of the *vas deferens* is at least five to seven times greater than the cirrus pouch, and replaces functionally a *vesicula seminis* which is lacking. Within the cirrus pouch the *vas deferens* may also be dilated, but never forming a distinct *vesicula seminis*. The *testes* occupy two narrow dorsal bands 0·17 mm. wide, situated in the lateral fields of the segment. In mature segments the testes number about 80 and measure from 0·05 to 0·07 mm. in diameter. They are situated to the right and to the left of the dorsal excretory vessels. In gravid segments in which the vessels are displaced, as already stated, they are situated inside of the latter. The *vasa efferentia* form a fine network.

The *Vagina* opens anterior to the cirrus pouch, and presents a slight *sphincter vaginae*; there is no distinct *receptaculum seminis*. The *ovary* forms in the posterior part of the segment a narrow band

just passing beyond the ventral excretory vessels. The ova possess very large nuclei nearly touching one another, so that the ovular plasma does not seem to be very abundant. The ovicapt is hardly developed, the shell gland is median, and is situated dorsally to the ovary. The *vitellaria* occupy two lateral bands situated within the

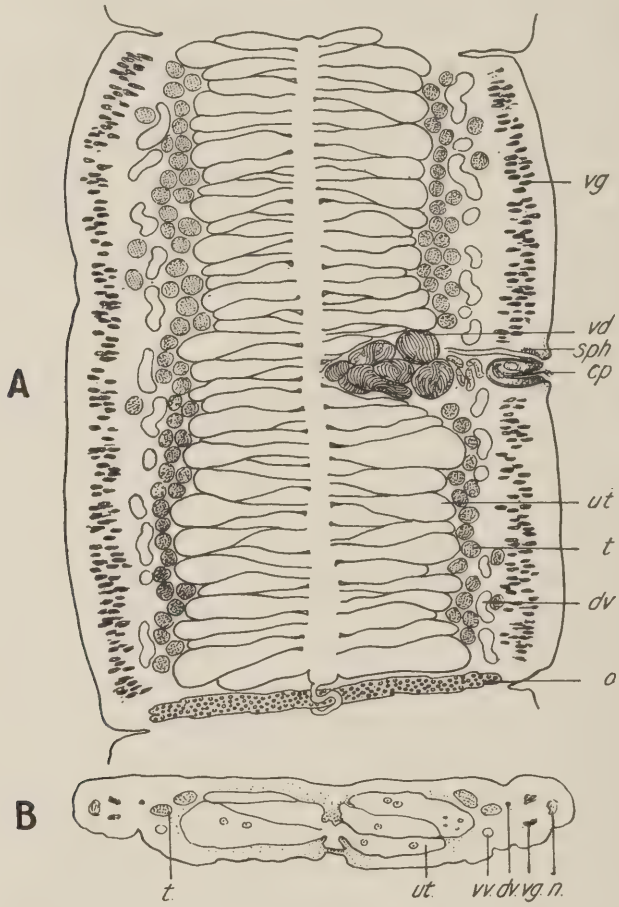


FIG. 2 A and B. *Ophiotaenia mönnigi* n.sp. A. Horizontal section of a mature segment. *cp.*—cirrus pouch; *dv.*—dorsal excretory vessel; *o.*—ovary; *sph.*—sphincter vaginae; *t.*—testes; *ut.*—uterus; *vd.*—vas deferens; *vg.*—vitelleria. B. Transverse section of mature proglottis. *n.*—longitudinal; *v.v.*—ventral excretory vessel. Other letters as A.

longitudinal nerve stems. The largest follicles are 0·12 mm. wide and 0·03 mm. long measured lengthways with regard to the segment. On transverse sections there are usually to be seen one dorsal and one ventral follicle (see fig. 2 B). The *uterus* is of the typical shape:

from a median stem fifty to fifty-seven diverticula, which are much longer than those of the other *Ophiotaenia* spp. (see fig. 2A), branch off on either side. On transverse sections we were able to observe that it is not a single diverticulum that branches off from the median stem, but at least two, and exceptionally three, diverticula all situated in the same dorso-ventral plane. Therefore, the number of diverticula indicated should be at least twice as great. We were unable to observe any uterine pores ; we have, however, observed on the ventral surface of the segments, a certain number of short, irregularly placed diverticula arising from the median stem, and reaching as far as the cuticula, where probably a rupture of the tissues allow the ova to escape. The embryos measure 0·012 to 0·013 mm. in diameter, and are surrounded by two envelopes, of which the exterior and thicker one is 0·03 mm. in diameter.

Our new species, although very characteristic, reminds one of the other South African species placed by Rudin (1917) in the *O. Theileri* group. This group contains the following species : *O. Theileri*, Rudin (South Africa), *O. Zschokkei*, Rudin (South Africa), both found in *Naja* spp., and *O. adiposa*, Rudin (Cameroon), and *O. gabonica*, Beddard (Gaboon) from *Bitis* spp.

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# ON A COLLECTION OF LINGUATULIDS IN THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

T. SOUTHWELL

(*Received for publication* 15 *October*, 1924)

The only feature of interest in this collection is the occurrence of several species of Linguatulids in fresh hosts and in new localities, the most important being the presence of *Porocephalus clavatus* in African snakes.

An attempt had been made to provide a key for all the known genera and species, but in the absence of adequate descriptions of numerous species the result is somewhat unsatisfactory. The keys are compiled from the descriptions given by Shipley and by Sambon in their respective papers, and the definitions of the various genera, sections, etc., are after Sambon. The only species I have been able to examine are pointed out in the text.

Except where otherwise stated, the measurements given refer to females only.

The Linguatulids are parasitic worm-like animals which are annulated in such a way that they resemble segmented worms. The annulations are, however, purely superficial for there is no segmentation of the genital organs such as exist in the Cestoda.

The systematic position of this family of parasites in the animal world is a matter on which some doubts exist, but the generally accepted opinion is that they are an aberrant, or degenerate branch of the Phylum Arthropoda and of the class ARACHNIDA (scorpions, spiders, etc.) and of the Order **Acarina** (mites and ticks) *i.e.*, that they are more or less related to ticks and mites, even though superficially they are worm-like in appearance. The limbs are represented by four hooks which encircle the mouth ; the muscle fibres are striated. Respiratory, circulatory and special sense organs are absent. The gut is a simple tube with no appendages ; the sexes are separate as in other Acarines and there is a small nerve ring which surrounds the oesophagus.

The synonymy of the parasites included in this family is very extensive. Frölich, in 1789, erected the genus *Linguatula* and included in it the species *serrata*. Humboldt (1811) established the genus *Porocephalus* to accommodate *P. crotali*. Rudolphi (1819) placed the species *taenioides, denticulatum, serratum, emarginatum* and *proboscideum* in a new genus which he named *Pentastomum*.

Within recent years the worms have been variously referred to as Linguatulids, Pentastomes and Porocephalids. It will be clear that the name Linguatula has priority, and this name is now used to designate the family.

Sambon (1922) defines the family LINGUATULIDAE as follows :—

'Blood sucking, endoparasitic *Acarina*. Body legless, elongate, vermiform, and more or less markedly annulated. Mouth round, squarish, or elliptical, provided with a chitinous armature of varied structure, and situated either before, behind or between two pairs of hollow, retractile, fang-like chitinous hooks emerging from four longitudinal pits or pouches disposed in trapezoidal formation or archwise. These hooks may be either equal or unequal, smooth or serrated, single or binate. Anus terminal or sub-terminal. Sexes divided ; female larger than male. Genital opening on mid-ventral line ; at anterior end of abdomen in male ; either at anterior or posterior end of abdomen in female. When at posterior extremity, the latter opens more or less near anus, but always anterior to it. The ovary is a long, dorsally-placed organ, usually extending almost the whole length of the abdomen. Anteriorly it divides into two oviducts, which bestride the alimentary canal, pass the spermathecae, receive the spermathecal ducts, and unite, forming either a wide egg-sac or a long tube, which combines the functions of both uterus and vagina. When tubular, the utero-vagina runs in a straight line to the posterior end in the virgin and permits copulation, but becomes greatly elongated in the gravid female, forming numerous and complex gyrations. Its coils may be amassed either above or below the alimentary canal, or they may be twined around it. Accordingly, the alimentary tube is either dorsal, ventral or axial. These anatomical details are given here because of their taxonomic importance. They are usually discernible in fully distended females owing to the transparency of the integument, but, in any case, they may be easily detected after clarification by the usual methods.

'The eggs are enclosed in a thin, bladder-like envelope filled with an albuminous substance clear as glass, and contain within their thick chitinous shell an oval embryo with rudimentary mouth parts and either six or four legs, each one tipped with two strong, claw-like hooks. On the back of the embryo may be seen the so-called dorsal organ or facette.'

Adult Linguatulids, although they occur occasionally in the gut or body cavity, are parasitic principally in the lungs of reptiles and in the frontal sinus and maxillary antra (connected with the nasal chambers) of the dog, the wolf and occasionally in man. They occur less rarely in birds and amphibians.

The larvae occur in animals which are preyed upon by the host in which the adult parasite lives.

In *Linguatula serrata* the eggs are passed in the discharge from the mouth and nostrils; they also occur in the faeces; each egg contains an embryo; when swallowed by the intermediate host the egg-shell is dissolved and the larva is liberated; the larva then bores its way to the liver, spleen, etc., and, after a series of moults it encysts, grows, and becomes a nymph. When this nymph is swallowed by the final host it makes its way from the gut to the lungs or the nasal cavities where it becomes adult; the nymph, when fully developed, is said to be able to leave the cyst and to migrate to the bronchi or to the intestine of the intermediate host, from which positions it is passed to the exterior. It reaches its final host by being sniffed up by the dog and becomes adult in the nasal cavities. It is also probable that, occasionally, the entire life-history is passed in one host.

Sambon (1922), in his Synopsis of the family LINGUATULIDAE, Parts I and II, classified the family as follows:—

Family LINGUATULIDAE, Shipley, 1898.

Sub-family 1. RAILLIETIELLINAE, Sambon, 1922.
Genera. *Raillietiella*, Sambon, 1910.
*Reighardia*, Ward, 1899.

Sub-family 2. POROCEPHALINAE, Sambon, 1922.

Section. *SEBEKINI*, Sambon, 1922.
Genera. *Sebekia*, Sambon, 1922.
*Alofia*, Giglioli, 1922.
*Leiperia*, Sambon, 1922.
*Sambonia*, Noc and Giglioli, 1922.
*Diesingia*, Sambon, 1922.

Section. *POROCEPHALINI*, Sambon, 1922.
Genera. *Porocephalus*, Humboldt, 1811.
*Kiricephalus*, Sambon, 1922.
*Armillifer*, Sambon, 1922.
*Waddycephalus*, Sambon, 1922.

Section. *LINGUATULINI*, Sambon, 1922.
Genera. *Linguatula*, Frölich, 1789.
*Subtriquetra*, Sambon, 1922.

KEY TO THE SUB-FAMILIES OF THE FAMILY LINGUATULIDAE.

Female genital opening anterior; mouth anterior to hooks. Salivary glands moderately developed; Larvae with six short legs..... *Raillietiellinae*

Female genital opening posterior; mouth in line with or posterior to hooks; salivary glands greatly developed. Larvae with four legs..... *Porocephalinae*

## Sub-family I. RAILLIETIELLINAE, Sambon, 1922.

*Diagnosis.*—LINGUATULIDAE:

'Female genital opening at anterior end of abdomen. Utero-vagina straight, ample, sacciform. Mouth anterior to hooks. Salivary glands moderately developed. Larva with six, short, stumpy legs.'

### Genus *Reighardia*, Ward, 1899.

*Diagnosis.*—RAILLIETIELLINAE:

'Body cylindrical, elongate, slightly attenuated at both ends. Integument covered with stud-like projections. Mouth in advance of hooks. Hooks exceedingly minute, placed in trapezoidal formation. Posterior extremity bluntly rounded and curved ventrally.

'Only one species known from Holarctic region. Adult form parasitic in air sac of Gulls and Terns (*Laridae*). Nymphal form probably encysted in fish.

'Type species: *Reighardia sternae* (Diesing, 1864), Ward, 1899.'

No specimens belonging to this genus were found in the collections of the Liverpool School of Tropical Medicine.

### Genus *Raillietiella*, Sambon, 1910.

*Diagnosis.*—RAILLIETIELLINAE:

'Body cylindrical, long, slender, flattened ventrally, tapering at both ends. Integument smooth, transparent. Mouth well in advance of hooks; opens on a terminal projection. Hooks simple, unequal, placed in trapezoidal formation; anterior pair smaller than posterior. Three vesicular projections about each hook, one anterior, globular, ensheathing the hook, the other two hemispherical, one on each side. Two vesicular projections placed dorsally on each side of cephalothorax, on a level with anterior pair of hooks. Posterior extremity bifid; terminal lobes divergent. Anus between terminal lobes.

'Six species described from Oriental, Ethiopian and Neotropical regions. Adult form parasitic in *Ophidia*, *Lacertilia* and *Bufonidae*.

'Type species: *Raillietiella boulengeri*, Vaney and Sambon, 1910.'

KEY TO GENERA AND SPECIES OF THE SUB-FAMILY RAILLIETIELLINAE.

Hooks exceedingly minute without vesicular projections; posterior extremity bluntly rounded..... *Reighardia* with one species only, viz., *R. sternae*

Hooks each with three vesicular projections; posterior extremity bifid, terminal lobes divergent..... *Raillietiella*

KEY TO SPECIES OF THE GENUS RAILLIETIELLA.

1. Each hook bears one or two stylets on its inner surface, close to base..... *R. geckonis*
   Stylets absent.....2
2. Worms with over 38 annuli.....6
   Worms with less than 38 annuli.....3
3. Small worms under 8 mm. in length..... *R. indica*
   Worms over 8 mm. in length.....4
4. None of the hooks are borne on parapodia..... *R. boulengeri*
   Either one or both pairs of hooks are borne on parapodia.....5
5. Posterior pair of hooks borne on long parapodia..... *R. mabuiae*
   Both pairs of hooks borne on long parapodia..... *R. tetrapoda*
6. Body of female spirally coiled..... *R. spiralis*
   Body of female not spirally coiled..... *R. furcocerca*

As I have been unable to discover any morphological differences between the following species they are considered identical, viz.,

*R. furcocerca.*
*R. furcocerca*, var. *orientalis* and
*R. furcocerca*, var. *mediterranea.*

*Raillietiella furcocerca* (Diesing, 1835), Sambon, 1910.

SYNONYMS :—*Pentastoma furcocercum*, Diesing, 1835.
*Pentastomum bifurcatum*, Diesing, 1850.
*Porocephalus bifurcatus*, Shipley, 1898.

One damaged specimen of what appears to be this species, obtained from the pericardial region of a Colubrine snake in Southern Nigeria (February, 1915), was presented by Dr. J. F. Corson.

*Raillietiella boulengeri*, Vaney and Sambon, 1910.

SYNONYM :—*Porocephalus boulengeri*, Vaney and Sambon, 1910.

Three densely gravid females from *Causus rhombeatus*. Accra, January, 1915. Collected and presented by Dr. J. W. S. Macfie.

Sub-family II. POROCEPHALINAE, Sambon, 1922.

*Diagnosis.*—LINGUATULIDAE :

'Female genital opening at posterior end of abdomen. Utero-vagina tubular, greatly elongated and forming numerous windings. Mouth in a line with, or posterior to, hooks. Salivary glands greatly developed, extending whole length of body on either side of alimentary tube. Larva with four legs.'

KEY TO SECTIONS OF THE SUB-FAMILY POROCEPHALINAE.

Body flattened..... *Linguatulini*
Body cylindrical.....1
1. Well-marked latero-ventral grooves; hooks in trapezoidal formation..... *Sebekini*
Latero-ventral grooves absent; hooks disposed archwise..... *Porocephalini*

## Section I. *Sebekini*, Sambon, 1922.

*Diagnosis.*—POROCEPHALINAE:

'Body cylindrical. Well-marked latero-ventral grooves. Hooks in trapezoidal formation. Alimentary canal dorsal, longer than body, sinuous.

'Type genus: *Sebekia*, Sambon, 1922.'

KEY TO GENERA OF THE SECTION SEBEKINI.

Female genital pore on fifth ring from posterior extremity..... *Sambonia*
Female genital pore on terminal ring.....1
1. Worms more or less spirally coiled..... *Leiperia*
Worms not spirally coiled.....2
2. Convex surface of hooks serrated..... *Sebekia*
Convex surface of hooks not serrated.....3
3. Body capsicum-shaped. Annulations limited to ventral surface *Diesingia*
Body not capsicum-shaped. Annulations complete..... *Alofia*

## Genus *Alofia*, Giglioli, 1922.

SYNONYMS:—*Pentastomum*, Lohrmann, 1889.
*Porocephalus*, Shipley, 1898.
*Reighardia*, Sambon, 1910.

*Diagnosis.—Sebekini:*

'Body small, massive, banana-shaped. Cephalothorax large, continuous with abdomen. Annuli 70-75. Mouth U-shaped, very large, with posterior margin on posterior hook-line and anterior end above anterior hook-line. Hooks comparatively large, single, equal and smooth. Alimentary tube largely sinuous. Utero-vagina ventral, convoluted. Anus terminal. Genital opening contiguous.

'One valid and two doubtful species described. Two from Samoa, the third unknown locality. Hosts unknown, probably fish.

'Type species: *Alofia ginae*, Giglioli, 1922.'

Four species of this genus have been recorded, but at present it is not possible to prepare a key to the species as the details relating to them are so scanty. No species of the genus occurs in the

collection of the Liverpool School of Tropical Medicine. The principal details relating to the four species are as follows :—

TABLE I.

| | Length | No. of annuli | Remarks |
|---|---|---|---|
| *A. ginae* ... | 15 to 20 mm. × 2 to 3 mm. | 75 | Chitinous oral armature U-shaped. |
| *A. merki* ... | 15 mm. × 3 mm. | 75 | |
| *A. platycephala* | 23 mm. × 2.8 mm. | 70 | |
| *A. adriatica* ... | 21 mm. to 85 mm. | 72 | Chitinous oral armature, key-shaped. |

## Genus *Sebekia*, Sambon, 1922.

SYNONYMS :—*Pentastoma*, Rud., 1819 (in part).
*Pentastomum*, Diesing, 1835.
*Porocephalus*, Stiles, 1893.
*Reighardia*, Sambon, 1910.

*Diagnosis.—Sebekini :*

'Body small, massive, closely annulated (annuli c. 80). Cephalothorax very small, wedge-shaped, projecting nipple-like from gross abdomen, ventral side continuous with that of abdomen. Mouth subterminal, shaped more or less like an inverted U, with free ends approximated. Hooks very small, distance between anterior and posterior hook-lines small. Hooks single, equal with convex surface serrated. Alimentary canal longer than body, forming sinuous loop about junction of anterior with medium third of body. Anus terminal. Utero-vagina long, much convoluted, amassed beneath alimentary tube. Ovary sinuous. Genital opening slightly anterior to anus.

'Six species described from Oriental, Ethiopian, Neotropical and Australasian regions. Adult forms parasitic in Crocodilians and Monitors. Nymphal forms probably in fish.

'Type species : *Sebekia wedli*, Giglioli, 1922.'

The details relating to the six species placed in this genus are so meagre that it is impossible to prepare a key. The principal point of differentiation between the species is said by Sambon to be the form of the chitinous oral armature surrounding the mouth. No species belonging to this genus were found in the collections of the Liverpool School of Tropical Medicine.

The following are the principal points relating to the six species described :—

TABLE II.

| | Length | No. of annuli | Remarks |
|---|---|---|---|
| *S. oxycephala* ... ... ... ? = *P. crocodili*, Wheeler ... | 10 mm. | 60 | |
| *S. divestei* ... ... ... | 10 mm. | 75 | |
| *S. wedli* ... ... ... | 10 to 25 mm. | 80 | |
| *S. cesarisi* ... ... ... | 15 mm. × 2 mm. | Not known | Each annulus bears a pair of digitate processes. |
| *S. indica* ... ... ... | 24 mm. × 5 mm. | Not known | |
| *S. jubini* ... ... ... | 42 mm. × 4.5 mm. | Not known | |

## Genus *Sambonia*, Noc and Giglioli, 1922.

*Diagnosis.—Sebekini :*

'Body incurved, tapering at both ends. Annulations (44 in type sp.), slightly imbricative, giving the body outline a serrated appearance. Cephalothorax small, wedge-shaped. Mouth and hooks close to anterior border. Mouth ovate, placed between hook lines with longest diameter vertical. Hook-trapezoid, low and relatively wide at base. Hooks simple, smooth, equal. Anus terminal. Female sexual opening above terminal segment (fifth ring from posterior end in type sp.). Ova with strikingly characteristic spined outer shell. Parasite of Lizards.

'Type species : *Sambonia lohrmanni* (Sambon, 1910), Noc and Giglioli, 1922.'

KEY TO SPECIES OF THE GENUS SAMBONIA.

| | Length | Annuli |
|---|---|---|
| *S. lohrmanni* ... ... ... | 13 to 17 mm. × 3 mm. | 44 |
| *S. wardi* ... ... ... ... | 75 to 150 mm. | 44 |

No specimens of this genus were found in the collections of the Liverpool School of Tropical Medicine.

## Genus *Leiperia*, Sambon, 1922.

*Diagnosis.—Sebekini :*

'Body large, cylindrical, elongate, more or less spirally coiled. Hooks in trapezoidal formation, simple, equal, smooth. Distance between hook-lines relatively great. Mouth small, situated on posterior hook-line. Utero-vagina beneath slightly sinuous alimentary tube. Posterior extremity tapering somewhat at the very end. Anus terminal. Female genital opening slightly anterior to anus.

'One species from Nilotic Crocodile (*Crocodilus niloticus*).

'Type species : *Leiperia cincinnalis* (Sambon, 1910), Sambon, 1922.'

The genus *Leiperia* contains one species only, viz., *L. cincinnalis.*

*Leiperia cincinnalis*, Sambon, 1922.

Very numerous young, adult, male and female specimens, all immature, from the lung of a crocodile. Upper Shire, Nyasaland. Collected by Professor R. Newstead, F.R.S., and Dr. Davey, in 1911.

The specimens measured from 35 mm. to 50 mm. in length and from 1 mm. to 1·3 mm. in breadth; the number of annuli varied from about 90 to 110. The female genital pore is situated on the last segment, slightly in front of the anus. The hooks are bifurcated, the larger branch measuring 212$\mu$ and the smaller 150$\mu$ in length. In the mature adult the hooks are simple.*

Genus *Diesingia*, Sambon, 1922.

*Diagnosis.—Sebekini:*

Body capsicum-shaped, the annulations being limited to the ventral surface; a median ventral groove is present.

*Type species.—D. kachugensis* (Shipley, 1910).

Sambon (1922) erected the genus *Diesingia* to accommodate *Porocephalus kachugensis*, Shipley, 1910, a capsicum-shaped nymph in which all the four hooks are bifid; it measured 12 mm. in length and 3 mm. in breadth, and had from 40 to 46 annuli; from *Kachuga lineata*, India.

Hett is of opinion that *D. kachugensis* is the larval form of *Subtriquetra megacephala* (Baird) and that, on account of its shape, etc., the latter species is intermediate between the genera *Linguatula* and *Subtriquetra*. If such proves to be the case then Baird's species will have to be referred to the genus *Diesingia*, and the latter genus to Sambon's section *Linguatulini*.

Section II. *Porocephalini*, Sambon, 1922.

*Diagnosis.—Porocephalinae:*

'Body cylindrical. No latero-ventral grooves. Hooks disposed archwise. Alimentary canal dorsal or axial, not longer than body, straight.

'Type genus: *Porocephalus*, Humboldt, 1811.'

* A number of smaller specimens of this species have since been obtained from 'a large fish,' Tanganyika.

KEY TO GENERA OF THE SECTION POROCEPHALINI.

The outer pair of hooks are bifurcated..... *Porocephalus*
Outer pair of hooks not bifurcated..... 1

1. Annulations marked by prominent bands giving the worm a beaded appearance..... *Armillatus*
Annulations not prominent.....2

2. Female pore opening considerably anterior to anus..... *Waddycephalus*
Female pore terminal..... *Kiricephalus*

## Genus *Porocephalus*, Humboldt, 1811.

SYNONYMS :—*Echinorhynchus*, Humboldt, 1808.
*Distoma*, Humboldt, 1808.
*Polystoma*, Rudolphi, 1812.
*Pentastoma*, Rudolphi, 1819.
*Linguatula*, van Beneden, 1849.
*Pentastomum*, Diesing, 1850.

*Diagnosis.—Porocephalini :*

'Body club-shaped ; posterior half curved more or less ventrally ; terminal segments dilated into characteristic olive-shaped enlargement. Annuli smooth. Cephalothorax bluntly rounded anteriorly. Hooks unequal ; inner simple, outer provided with non-caducous accessory spine ; disposed in slightly arcuate line with convexity posterior. Mouth ovate, placed on inner hook-line. Anus subterminal. Female genital opening placed on terminal segment anterior to, and continuous with, anus. Alimentary canal axial, short, straight. Utero-vagina twined around alimentary tube. Four valid species described from Ethiopian and Neotropical regions. Adult forms parasitic in Ophidians ; nymphal forms encysted in mammals. Probably attacks man also.

'Type species : *Porocephalus crotali*, Humboldt, 1811.'

KEY TO SPECIES OF THE GENUS POROCEPHALUS.

Over 70 annuli..... *P. crotali*
Less than 70 annuli.....1

1. Annuli quite distinct.....2
Annuli indistinct and from 1 to 2 mm. apart..... *P. subulifer*

2. With 35 to 43 annuli..... *P. clavatus*
With 45 to 50 annuli..... *P. stilesi*

It should be noted here that the identity of *P. crotali*, Humboldt, is not definitely established. Hett (1924, pp. 141-144) discusses the question at some length. Humboldt obtained the species from a rattlesnake (*Crotalus cumanensis*) but did not state how many annuli the worm possessed. Diesing (1835 and 1850) described this and several other species under the name *Pentastomum proboscideum*, including one form from a boa and another only recorded hitherto from *Lachesis* spp. According to Diesing, the number of annuli is 80, but in his figures a varying number are shown. Leuckart examined Diesing's material

and stated that the worms possessed only about 40 annuli. Sambon accepts Diesing's statement that *P. crotali* has 80 annuli, but Hett (1924) agrees with Leuckart, and describes *P. crotali* as possessing about 40 annuli.

*Porocephalus clavatus* (Wyman, 1845), Sambon, 1910.

SYNONYMS :—Adult : *Linguatula clavata*, Wyman, 1847.
*Linguatula proboscidae*, van Ben., 1849, in part.
*Pentastomum proboscideum*, Leidy, 1856, in part.
*Pentastomum clavatum*, Leuckart, 1860.
*Pentastomum imperatoris*, Macalister, 1875.
*Pentastoma moniliforme*, Mégnin, in part.
Nymph : *Pentastomum didelphidis virginianae*, Leidy, 1852.

Specimens were obtained from the following sources :—(1) Two gravid females from a snake (genus and species unknown) : Leverville, Congo, October, 1923 ; collected by Dr. Brassart. (2) One specimen from a snake ; species and locality unknown. (3) Three specimens from a snake (*Causus rhombeatus*) : Gold Coast, September, 1915 : collected and presented by Dr. Corson.

This species has hitherto only been recorded from *Boa constrictor* and *Boa imperator*.

Most species of true boas inhabit the warmer parts of Central and Southern America, but two species occur in Madagascar.

*Porocephalus subulifer* (Leuckart, 1860), Stiles, 1893.

SYNONYM :—*P. cercopitheci*, Breinl and Hindle, 1908.

Several specimens from the lung of a Green Guenon (*Cercopithecus callitrichus*).

Genus *Kiricephalus*, Sambon, 1922.

SYNONYMS :—*Pentastomum*, Diesing, 1850.
*Porocephalus*, Shipley, 1898.

*Diagnosis.—Porocephalini :*

' Body club-shaped (like the Kaffir " kiri " and other knobbed clubs, sometimes armed with spikes), greatly elongate, of uniform thickness throughout and spirally twisted on its own axis. Annuli smooth. Cephalothorax more or less globular, owing to the constriction of anterior body rings brought about by habit of inserting cephalothorax deeply into host's lung. Hooks slightly unequal in size, but simple, without accessory spines. Mouth ovate, placed just below inner hook-line. Anus sub-terminal. Female sexual opening placed on terminal segment, somewhat anterior to anus. Alimentary canal axial ; on account of body torsion, appears to twine around utero-vaginal skein.

'Three valid species described from Oriental, Neotropical and Australasian regions. Parasitic in Ophidia.

'Type species : *Kiricephalus coarctatus* (Diesing, 1850), Sambon, 1910.'

KEY TO SPECIES OF THE GENUS KIRICEPHALUS.

| | Length | Annuli |
|---|---|---|
| *K. coarctatus* ... ... | ... 76 to 115 × 4 mm. | 52 |
| *K. pattoni* ... ... ... | ... 80 to 115 × 2·5 mm. | 36 |
| *K. tortus* ... ... ... | ... 40 mm. | 25 |

## *Kiricephalus pattoni* (Stephens, 1908), Sambon, 1922.

SYNONYM :—*Porocephalus pattoni*, Stephens, 1908.

The type specimens of this species from *Zamenis mucosus* are in the collections of the Liverpool School of Tropical Medicine. (1) Four females and one male were also obtained from a snake (species unknown) : Hong Kong ; presented by Dr. Bell. (2) Two females and one male from *Zamenis mucosus :* Zoological Gardens, Calcutta ; presented by Captain Knowles, I.M.S., 27.3.1923.

## Genus *Armillifer*, Sambon, 1922.

SYNONYMS :—*Pentastoma*, Diesing, 1835.
*Linguatula*, Wyman, 1847.
*Nematoideum*, Diesing, 1851.
*Pentastomum*, Harley, 1856.
*Porocephalus*, Stiles, 1893.

*Diagnosis.—Porocephalini :*

' Body cylindrical, elongate, slightly curved ventrally ; terminal segment conical. Annulation strongly marked by thick, prominent bands in each segment, giving the species of this genus a beaded or ringed window-pole appearance. Cephalothorax wedge-shaped, with anterior border rounded. Hooks robust, equal, simple ; placed in straight or slightly arcuate line. Mouth orbicular, placed just above inner-hook line. Anus terminal. Female genital opening on terminal segment, somewhat anterior to anus. Alimentary tube dorsal, short, straight. Utero-vaginal coils amassed beneath alimentary tube. Three valid species described from Ethiopian and Oriental regions.

' Adult forms : Parasitic in Ophidia, nymphal forms in mammals and birds.

' Type species : *Armillifer armillatus* (Wyman, 1847), Sambon, 1922.'

KEY TO SPECIES OF THE GENUS ARMILLIFER.

With a neck-like constriction between cephalothorax and body ..... 3
Neck-like constriction absent ..... 1
1. With 28 to 35 annuli ..... *A. moniliformis*
With less than 28 annuli ..... 2
2. With two small papillae in front of mouth ..... *A. armillatus*
With a single small lobe in front of mouth ..... *A. grandis*
3. Neck about 1 mm. in length ..... *A. annulatus*
Neck 5 to 7 mm. in length ..... *A. pomeroyi*

Sambon considers that *Porocephalus pomeroyi*, Woodland, is identical with *A. annulatus ;* but Hett believes them to be distinct species and gives a table showing the differences between the two forms.

*Armillifer moniliformis* (Diesing, 1835).

SYNONYMS :—Adult : *Pentastoma moniliforme*, Diesing, 1835.
*Pentastoma moniliforme*, Leuckart, 1860.
*Linguatule moniliforme*, Mégnin, 1880.
*Porocephalus moniliformis*, Stiles, 1893.
Nymph : *Pentastomum tornatum*, Creplin, 1849, in part.
*Pentastomum aonycis*, Macalister ,1874.
*Porocephalus armillatus*, Stiles, 1908, in part.

Five female specimens (gravid) from *Tropidonotus picturatus*, Darwen, Northern Territory, Australia. Presented by Dr. P. A. Maplestone, D.S.O., M.B., Ch.B., Australia.

The adult form has hitherto only been recorded from the Indian python which inhabits India, Ceylon and Indo-China.

*Armillifer armillatus* (Wyman, 1847), Sambon, 1922.

SYNONYMS :—Adult : *Linguatula armillata*, Wyman, 1847.
*Pentastomum polyzonum*, Harley, 1856.
*Pentastomum amillatum*, Leuckart, 1860 (misprint).
*Pentastoma armillata*, Wyman, 1863.
*Pentastomum armillatum*, Diesing, 1864.
*Porocephalus armillatus*, Stiles, 1893.
*Porocephalus polyzonus*, Stiles, 1893.
*Porocephalus moniliformis*, Neumann, 1899 (in part).
Nymph : *Linguatula diesingii*, van Ben., 1849.
*Pentastomum tornatum*, Creplin, 1849 (in part).
*Pentastomum euryzonum*, Diesing, 1850.
*Nematoideum hominis* (*viscerum*), Diesing, 1851.
*Pentastomum constrictum*, Von Siebold, 1852.
*Linguatula constricta*, Küchenmeister, 1855.
*Pentastoma leonis*, Wedl, 1863.
*Pentastomum leonis*, Diesing, 1864.
*Pentastoma tornatum*, Cobbold, 1879.
*Pentastomum protelis*, Hoyle, 1883.
*Porocephalus constrictus*, Stiles, 1893.
*Linguatula constrictor*, Galli Valerio, 1896 (misprint).
*Pentastomum diesingii*, Shipley, 1898.

Specimens were examined from the following sources :— (1) Several nymphs from spleen, lungs, liver, etc., of man : Freetown, Gold Coast, West Africa ; collected by Dr. S. Adler, 18.12.1923. (2) Four specimens, nymphs, from liver of man ; presented by Dr. Manuk, Lokoja. (3) Two specimens from a dead prisoner ;

presented by Dr. J. Hannington, M.O., Nigeria. (4) Six specimens, immature adults, from a Kroo Boy: Degama, Gold Coast, July, 1913; Dr. H. A. Wilson; collected and presented by Dr. J. W. S. Macfie. (5) Two nymphs from man, Akoada District, E.P. Nigeria, 13.9.13.; collected by Dr. H. A. Wilson and presented by Dr. J. W. S. Macfie. (6) One nymph from man: Accra, Gold Coast; collected by Dr. Findley, and presented by Dr. J. W. S. Macfie. (7) Several nymphs from liver of man, and one from mesentery: Accra, Gold Coast; collected and presented by Dr. J. W. S. Macfie. (8) Several nymphs from man: Accra Gold Coast, October 15, 1916; collected and presented by Dr. J. W. S. Macfie. (9) Two specimens from the peritoneal cavity of a chimpanzee; presented by Mr. Walker, 22.12.06. (10) One gravid female from a pig's liver: Accra, Gold Coast; collected and presented by Dr. J. W. S. Macfie, 12.3.1923. (11) Three nymphs from liver of an ox: Accra, Gold Coast; collected and presented by Dr. J. W. S. Macfie. (12) Several nymphs from liver of cattle: Accra, Gold Coast, 21.1.21; collected and presented by Dr. J. W. S. Macfie. (13) Very numerous adult specimens from a hedgehog: Accra, West Africa, 1.12.22; collected and presented by Dr. J. W. S. Macfie. (14) One adult female specimen measuring 80 mm. in length from lung of *Bitis masicornis*, Kumasi. (15) Three very large females from intestine of a horned *Cerastes*, Kumasi; presented by Dr. Tweedy.

## Genus *Waddycephalus*, Sambon, 1922.

Synonyms:—*Pentastoma*, Baird, 1862.
*Pentastomum*, Spencer, 1898.
*Porocephalus*, Shipley, 1898.

*Diagnosis.—Porocephalini:*

'Body club-shaped (like Australian "waddy"), tapering considerably towards posterior extremity, which ends in bilobed segment. Annuli smooth. Cephalothorax somewhat rounded, owing to constriction of anterior body segments. Hooks simple, unequal, inner larger, outer smaller, placed in arcuate line, the outer not only above the inner, but somewhat laterally. Mouth cordate, placed on inner hook-line. Anus between terminal lobes. Female genital opening considerably anterior to anus, placed on eighth body ring in *W. teretiusculus*. Alimentary canal axial, coils of utero-vagina amassed beneath alimentary canal.

'One species only described by Baird, in 1860, and again more fully by Spencer in 1893. Found in Australian snakes.'

KEY TO SPECIES OF THE GENUS WADDYCEPHALUS.

| | Length | Annuli | Pore |
|---|---|---|---|
| *W. teretiusculus* | ... 60 mm. × 5 mm. | Female 65 to 76 (50 to 70 Hett) Male 88 (60 Hett). | On 8th ring from anus |
| *W. mazzai* | ? | 41 to 44 | On 3rd ring from anus. |

*Waddycephalus mazzai*, Sambon, 1922.

SYNONYM :—*Pentastomum moniliforme*, Mazza, 1898.

Several adult female specimens from a snake, species unknown : Hong Kong ; presented by Dr. Bell.

Two species of this genus are known, viz., *W. teretiusculus*, found in the lungs of Australian snakes and *W. mazzai*, the host of which is unknown.

The specimens from Hong Kong agree in detail with the descriptions of *W. mazzai*.

## Section III. *Linguatulini*, Sambon, 1922.

*Diagnosis.—Porocephalinae :*

'Body flattened fluke-like, more or less convex in middle part of dorsal surface, sides depressed. Hooks disposed archwise. Alimentary canal axial. Utero-vagina twines around it.

'Type genus : *Linguatula*, Frölich, 1789.'

KEY TO GENERA OF SECTION LINGUATULINI.

Body elliptical, flat ventrally, arched dorsally..... *Subtriquetra*
Body spatulate, attenuated posteriorly..... *Linguatula*

## Genus *Subtriquetra*, Sambon, 1922.

SYNONYMS :—*Pentastoma*, Bresner, 1824.
*Pentastomum*, Diesing, 1850.
*Linguatula*, Railliet, 1883.

*Diagnosis.—Linguatulini :*

'Body more or less elliptical, flattened ventrally, greatly prominent dorsally. Adult form : Parasitic in crocodilians. Nymphal form : in fish.

'Type species : *Subtriquetra subtriquetra* (Diesing, 1835), Sambon, 1922.'

KEY TO SPECIES OF THE GENUS SUBTRIQUETRA.

Hooks simple..... *S. subtriquetra*
Hooks with subsidiary spines..... *S. megacephala*

The description of *Subtriquetra shipleyi*, Hett, 1924, is so meagre that it is impossible to distinguish it from *S. subtriquetra*.

Baird described the hooks of *S. megalocephala* as simple ; Sambon

states that they have accessory spines; but Hett, who examined the types, was unable to find accessory spines.

It has already been pointed out that it appears probable that *S. megacephala* is an intermediate form between the genera *Subtriquetra* and *Linguatula*. If Hett's opinion is correct, and it seems probable, then *S. megacephala* will have to be referred to the genus *Diesingia*, and the latter genus to the section *Linguatulini*, Sambon.

No specimens of this genus were found in the collections of the Liverpool School of Tropical Medicine.

## Genus *Linguatula*, Frölich, 1789.

SYNONYMS :—*Taenia*, Pilger, 1803.
*Halysis*, Zeder, 1803.
*Cochlus*, Rudolphi, 1805.
*Prionoderma*, Rudolphi, 1808.
*Polystoma*, Rudolphi, 1809.
*Echinorhynchus*, Braun, 1809.
*Tetragulus*, Bosc., 1810.
*Linguatula*, Lamarck, 1816.
*Pentastoma*, Rudolphi, 1819.

*Diagnosis.—Linguatulini :*

'Body spatulate, attenuated posteriorly. Cephalothorax anteriorly obtuse. Mouth sub-terminal, squarish, situated between inner hooks. Hooks simple, equal, disposed archwise. Alimentary canal straight. Anus terminal. Uterus anteriorly twined round alimentary tube. Parasitic in mammals.

'Type species : *Linguatula serrata*, Frölich, 1789.'

KEY TO SPECIES OF THE GENUS LINGUATULA.

| | Length | Posterior end |
|---|---|---|
| *L. serrata* ... ... | ... 80 to 130 mm. | simple |
| *L. recurvata* ... ... | ... 13 to 27 mm. | bifid and curved dorsally |

## *Linguatula serrata*, Frölich, 1789.

SYNONYMS :—Adult form.—*Ténia lancéolé*, Chabert, 1787.
*Ver rhinaire*, Chabert, 1787.
*Taenia rhinaris*, Pilger, 1803.
*Taenia lanceolata*, Rudolphi, 1805.
*Cochlus rhinarius*, Rudolphi, 1805.
*Prionoderma rhinarium*, Rudolphi, 1808.
*Polystoma taenioides*, Rudolphi, 1809.
*Taenia rhinaria*, Rudolphi, 1810.
*Linguatula taenioides*, Lamarck, 1816.
*Prionoderma lanceolata*, Cuvier 1817.
*Pentastoma taenioides*, Rudolphi, 1819.
*Linguatula lanceolata*, de Blainville, 1828.
*Linguatula rhinaria*, Railliet, 1900.

Nymph : *Linguatula serrata*, Frölich, 1789.
*Taenia capraea*, Abildgaard, 1789.
*Taenia caprina*, Gmelin, 1800.
*Polystoma serratum*, Goeze, 1803.
*Halysis caprina*, Zeder, 1803.
*Linguatula denticulata*, Rudolphi, 1805.
*Echinorhynchus caprae*, Braun, 1809.
*Polystoma denticulatum*, Rudolphi, 1809.
*Tetragulus caviae*, Bosc., 1810.
*Pentastoma denticulatum*, Rudolphi, 1819.
*Pentastoma serratum*, Rudolphi, 1819.
*Pentastoma emarginatum*, Rudolphi, 1819.
*Pentastoma fera*, Creplin, 1829.
*Pentastoma taenoides*, Dick, 1840 (misprint).
*Linguatula ferox*, Gros, 1849.
*Linguatula caprina*, R. Blanchard, 1895.
*Linguatula rhinaris*, Railliet, 1900.

(1) One specimen from a dog's nose ; presented by Captain Carter, I.M.S., India. (2) A number of nymphs from the liver of a male bushbuck (*Tragelaphus scriptus*) : Upper Shire, Nyasaland ; collected and presented by Professor R. Newstead, F.R.S., and Dr. Davey ; 1911. (3) One specimen from the nose of a dog : Manchester, England ; lent by A. W. Noel Pillers, Esq., F.R.C.V.S., D.V.S.M. Mr. Pillers states that he has only obtained this parasite twice in twenty years in England, once as noted above, and a second specimen from the nasal cavity of an otter hound in Shropshire.

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# NOTES ON SOME AFRICAN CERATOPOGONINAE—SPECIES OF THE GENUS *FORCIPOMYIA*

BY

A. INGRAM

AND

J. W. S. MACFIE

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| " *melanchroa* sp.n. | 591 |
| " *nigeriensis* sp.n. | 592 |

The collections of Ceratopogonine midges made by us at Accra, mostly between the years 1919 and 1922, contained very numerous specimens of *Forcipomyia ;* indeed, certain species of this genus (*e.g.*, *F. castanea*, *F. ingrami*, and a species allied to *F. chrysolopha*) were amongst the most abundant in the laboratory where the majority of our specimens were obtained. The task of examining so large a number of specimens has been no light one and for unavoidable reasons has only been completed recently, the results being embodied in the following paper. In addition to our Accra collections we have had at our disposal a small number of specimens from other places in the Gold Coast and from Nigeria, and we have been

privileged, thanks to the kindness of Dr. G. A. K. Marshall, to examine a small collection of *Forcipomyia* belonging to the Imperial Bureau of Entomology. This collection, which is largely composed of specimens taken by ourselves before we began our investigation, contains specimens from various parts of East and West Africa, and although not including any species not previously identified in our own collections, extends in several cases the range of distribution.

The large number of specimens at our disposal has enabled us to study in a few species the important question of the range of variation, with results which are interesting although rather disconcerting. We have found, for example, that such a character as the presence of bands on the legs is liable to great variation, and that colour markings are sometimes quite unreliable as specific characters. In a long series of specimens of *F. ingrami* we have found the general colouring to vary from very dark brown to quite a pale, yellowish-brown ; in *F. castanea* the characteristic sepia-brown band on the hind femora may vary considerably in size and depth of colour, may be reduced to a mere spot, or may be entirely absent ; and in *F. inornatipennis* the legs may be uniformly coloured, or may show dark bands on the hind femora only, on the hind femora and tibiae, or on both middle and hind legs. On the other hand, identical colour markings may be no sure indication of specific identity, for from a long series of specimens somewhat resembling *F. chrysolopha*, we have separated males of three species with quite dissimilar genitalia which we are unable to distinguish by any other characters. Such observations inevitably shake confidence in specific differentiations based mainly or entirely on colour markings, and make it difficult or impossible to estimate the value of descriptions, written perhaps many years ago, at a time when morphological characters, especially those of the genitalia, were either ignored or referred to but briefly. In the present state of knowledge it appears impossible to tell whether certain species of *Forcipomyia* are really different or differ only in description, and no decision can be reached without a re-examination of the type specimens.

In the descriptions of species which follow, we have given a somewhat detailed account of *F. castanea*, because we have in our possession abundant adult specimens of this species, both males and

females, and also pupae, larvae, and eggs, and have curtailed so far as possible the descriptions of the other species. The unit frequently referred to in the text is $3{\cdot}7\mu$. All measurements were made from specimens immersed in pure carbolic acid, and thus restored to their natural size and not shrunken as they tend to be when dry. The midges vary considerably in size, the ratios of the measurements are therefore probably of greater value than the actual measurements themselves.

The types and co-types of the new species described have been deposited in the collection of the Liverpool School of Tropical Medicine.

In some of our previous papers we have given keys for the identification of the Gold Coast species of certain genera of Ceratopogoninae. In this paper we venture to go a step further and have attempted a key for the identification of the African species of the genus *Forcipomyia*. We have done this, because it is now known that many Ceratopogonine midges are widely distributed in Africa (*e.g. Culicoides schultzei* in East, South, and West Africa, and in Egypt) and it is probable that, as time goes on, many more will be found to extend over a large part of the Continent, including regions with dissimilar climates. Some species, indeed, appear to have an even more extensive range of distribution, and although it is certainly artificial to make a limit of the shores of the Mediterranean, the Suez Canal, and the Red Sea, it appears to us that no grouping would be entirely satisfactory which did not include all known species (a project at present impracticable), but that in the meantime a key to the African species would be useful.

In drawing up our key we have used primarily the following characters in order :—the presence or absence of scales, the relative lengths of the first and second tarsal segments of the hind legs, the presence or absence of pale coloured spots on the wings, the adornment of the legs, and the form of the antenna of the females. They are all characters readily seen with a hand-lens or low-power microscopic objective, excepting the first, which we felt compelled to use as it has been made a generic character by Kieffer and certainly appears to separate off a somewhat compact group of species, which we regret is not always easy to make out in dry specimens. We have encountered great difficulties, to some of which we have already

referred. Unfortunately, also, only one sex is known of many species; the genitalia of males (which furnish most valuable differential characters) are often described imperfectly, as seen with a hand lens; there is often doubt as to whether descriptions are adequate in the case of important characters (such as the presence of scales) not easily determined at low magnifications; and with the exception of the species described below and two others from Egypt previously described by one of us, we have had no opportunity of augmenting the published descriptions by reference to the insects themselves. Our key, therefore, can only be regarded as tentative. It almost certainly includes as different species several which are actually identical.

### GENUS *FORCIPOMYIA* (Megerlé, 1818) Kieffer, 1901

External Morphology.

Adults. Cuticle all over the body covered by minute upright microtrichia. *Head.* Eyes usually bare; but the cuticle between the eyes and the bases of the antennae is covered by minute hairs, and a very few similar hairs may be found occasionally between the facets at the margin of the eyes; no account is taken, however, of such hairs in the descriptions of species which follow. Eyes usually broadly contiguous above in both sexes, the facets separated by a narrow line. Vertex and occiput, in both sexes, bearing numerous, stout, forwardly-projecting hairs, and sometimes scales also. Immediately in front of the anterior border of the eyes is a conical thickening of the cuticle or callosity which is sometimes prominent. Clypeus pronounced, especially in females, hairy.

*Mouth-parts.* Proboscis rather longer than the head in females, shorter in males. The component organs generally similar to those of *Culicoides* (see *F. castanea*), mandibles armed with numerous small teeth, maxillae with fewer, delicate, widely-spaced teeth. Palpi in both sexes composed of five segments, bearing moderately long and short hairs. Second, fourth, and fifth segments usually about sub-equal, about twice as long as broad. The third segment the largest, usually as long as the fourth and fifth together, inflated about the middle or basally, in some species very greatly, and containing a sub-spherical or oval sensory pit opening on the inner

aspect, in which are a number of minute, modified, drumstick-like, sensory hairs. In some species modified sensory hairs are present also on the surface of the third segment on its inner aspect. The fifth segment only slightly if at all expanded at its end. In the males the palpi are usually more slender than in the females, and the third segment inflated to a lesser degree and furnished with a smaller sensory pit.

*Antennae.* Composed of fifteen segments, not sculptured ; pilose in the female, plumose in the male. In the female the first segment small, bearing stout hairs which are directed anteriorly and are sometimes pubescent. Torus sub-spherical, bearing a few small hairs. Third segment sub-spherical or oval, larger than the fourth, with a posterior stalk of variable length. Segments four to ten usually sub-spherical to oval, sometimes flask-shaped, becoming progressively longer and narrower towards the tenth ; segments eleven to fifteen sometimes forming an almost continuous series with the basal segments, sometimes elongated, sub-cylindrical, an abrupt change of shape taking place between the tenth and eleventh segments. The combined length of segments 3 to 10 usually greater than that of segments 11 to 15. The last segment is the longest and ends in a blunt, often club-shaped, stylet. Hairs on the basal segments short, about twice the length of the segments or less, arranged in whorls of frequently about a dozen or more hairs. Hairs on the five distal segments usually rather shorter, those arising posteriorly the longest, forming a whorl, the rest scattered. All the flagellum segments bear small, colourless spines ; the basal segments bear also longer spines which are usually curved or geniculate, and may be very stout and either pointed or blunt. In the male the first segment devoid of hairs. Torus very large, shaped like a Dutch cheese hollowed out in the middle anteriorly, without hairs. Third segment sub-spherical, smaller than the fourth, from which it is separated by a wide membranous interspace, with a rather long stalk. Segments four to eleven from sub-spherical to irregularly ovate, broadly united, gradually becoming narrower and more drawn out anteriorly towards the eleventh ; segments twelve to fifteen elongated, sub-cylindrical, sharply separated from one another, the twelfth usually much the longest and shaped like the eleventh, but with the distal end greatly extended, the relative lengths of the other three varying

in different species. The last segment is broader than the others and ends in a blunt, often club-shaped stylet. Large spines are present on the basal, and smaller spines on all the segments of the flagellum ; and on segments four to twelve are large whorls of hairs, transverse on the basal segments but oblique on the more distal ones, forming a plume which reaches nearly to the level of the end of the antenna. The whorl on segment three is reduced ; on segments thirteen to fifteen are numerous shorter hairs, those at the bases of segments thirteen and fourteen arranged as small whorls.

*Thorax* strongly arched, but not projecting anteriorly over the head. Thoracic pits absent. Dorsum clothed with short hairs and sometimes scales, and bearing laterally and posteriorly a few longer, stouter hairs, some of which in some species form a curved row on each side a little anterior to the scutellum. Dorsum often adorned with three broad longitudinal stripes separated by narrow, paler-coloured lines, the middle stripe deficient posteriorly, and the lateral ones deficient anteriorly ; more laterally there is often an additional, small, narrow, longitudinal stripe on each side. Scutellum a transverse strip of chitin with a concave anterior and convex posterior border ; bearing numerous bristles and hairs and sometimes scales. Post-scutellum devoid of hairs.

*Wings* usually unadorned, but sometimes with one or more pale spots. Surface of the wings entirely covered with minute microtrichia which vary in size in different species to some extent, but are smaller than those of *Atrichopogon ;* and more or less densely clothed with larger, decumbent hairs or scales. The decumbent hairs are more or less lanceolate and curved, less straight and rigid-looking than in *Atrichopogon* and not tapering so regularly to sharp points; they are sometimes sub-plumose. The hairs are usually denser and darker on the anterior part of the wing, but there is a small area in this region, immediately distal to the end of the costa, which is bare. There are no bare areas along the veins as in *Atrichopogon.* The fringe on the posterior border of the wing is long, composed of one or two rows of long hairs between two rows of shorter, oblique hairs—the hairs lanceolate, angled or curved, and sometimes pubescent or even sub-plumose. The wing of the female is broad, in most species broadest near the base, with a rounded tip and a well-formed anal

angle ; that of the male is longer and narrower, and usually much paler in colour. The costa reaches to about the middle of the wing. The first and third veins are more or less fused basally, the first radial cell being completely absent or reduced to a mere slit, visible only with the aid of staining reagents. Distal radial cell well-formed, generally triangular, but varying in size and shape in different species. Radio-medial cross-vein oblique. The distance from the cross-vein to the base of the fifth vein greater than the distance from the cross-vein to the end of the costa. Fourth vein with a short petiole, about the same length as the cross-vein ; rami long, more or less obsolete proximally. Fifth vein forking near the level of the end of the costa, either a little distal to it or a little proximal to it ; anterior ramus continuing the line of the stem ; in most species both rami reaching the margin of the wing at a level distal to that of the end of the costa. Intercalary veins well-developed ; that between the end of the costa and the tip of the wing Y-shaped.

*Legs* often adorned with darker bands or spots on the femora and tibiae, and tarsal segments frequently infuscated ; somewhat densely clothed with hairs which are sometimes very long, and in certain species bearing also scales. Femora not greatly swollen, unarmed, but occasionally bearing hastate spines. Tibiae not greatly swollen ; occasionally bearing hastate spines. Fore tibiae armed at the distal extremity with a stout spur, a patch of strong, spine-like hairs, and an oblique transverse row of short, fine bristles ; middle tibiae usually unarmed, but a few bristles in a transverse row near the apex are highly developed and may be of great length ; hind tibiae with a stout spur and two transverse rows of graded bristles apically which are similar to those of *Culicoides*, but composed of more numerous elements. Tarsal segments with apical bristles on the first four segments differentiated, spine-like ; and a more or less complete longitudinal row, or rows, of similar differentiated bristles on the three first segments. Fifth tarsal segment unarmed. All the tarsal segments cylindrical ; the first may be longer or shorter than the second or of equal length, the third, fourth, and fifth progressively diminishing in length. Claws equal, about half the length of the last tarsal segment, sharply curved ; in the female simple, in the male usually with bifid ends, and more delicate and more sharply curved than in the female.

Empodium about as long as the claws, with long hairs on both lateral margins.

*Abdomen* short and broad in the female, longer and more slender in the male ; not petiolate. Hypopygium of the male large and conspicuous. Lamellae of the female small and rounded. Well-clothed with hairs and sometimes scales. Spermathecae usually two in number, highly chitinised, oval or pyriform in most species ; the commencement of the duct often chitinised for a short distance.

*External genitalia of the male.* The hypopygium is relatively large and complex, and presents morphological characters which are of value in distinguishing closely-allied species, and in some cases indeed have been the only structures in which we have been able to detect specific differences. In describing the various structures composing the hypopygium we have adhered to the nomenclature used in our previous papers.

The *ninth segment* is well-chitinised, often somewhat narrowed anteriorly, so that the tergite which is narrowed posteriorly also, has a shape suggestive of the ' lozenge ' ; both tergite and sternite usually well-clothed with moderately long hairs. Sternite usually not notched in the middle line posteriorly. Tergite relatively short, prolonged posteriorly as a membranous extension bearing dorsally a pair of long, stout hairs, and terminating laterally in small, irregularly-shaped processes which are often slightly chitinised and bear usually two long and two short hairs. *Forceps* well-developed, highly chitinised. Side-pieces large, oval, densely clothed with hairs, some of which are often of great length. Dorsal root-like process long and slender, highly chitinised, often appearing to be jointed at its base. Claspers about as long as the side-pieces, usually less highly chitinised, broad at the base and narrowing rapidly towards the apex ; clothed at the base at least with minute hairs intermixed with which are a few larger hairs, and bearing at the apex a few minute hairs. *Harpes* usually in the form of two chitinised admedian plates or rods which project posteriorly and slightly ventrally, may or may not be joined anteriorly across the middle line by a strip of chitin, and articulate by their proximal ends with the distal ends of the dorsal root-like processes at the bases of the side-pieces. In some species, however, no such posteriorly projecting structures are present, and the dorsal root-like

processes at the bases of the side-pieces join across the middle line anteriorly. The harpes vary in shape and size in different species, and are of great systematic value. *Aedoeagus* a complicated structure, more or less conical, triangular, shield-shaped, or stirrup-shaped in ventral-view, and largely membranous or but feebly chitinised. The aedoeagus varies greatly in shape in different species and is of value in distinguishing them.

PUPA AND LARVA. A detailed morphological study of the pupa and larva of *Forcipomyia* has recently been published by Saunders. As we succeeded in obtaining materials illustrating the early stages of only two species, and as Saunders states that he expects shortly to publish descriptions of the early stages of further, exotic species, it is only necessary for us to refer here to his work.

## KEY TO THE AFRICAN SPECIES OF THE GENUS *FORCIPOMYIA*

1. With scales (*Lepidohelea*) ..... 2
   Without scales .....15

2. First tarsal segment of hind legs longer than second*..... 3
   First tarsal segment of hind legs shorter than second or of about equal length..... 4

3. Legs entirely brown ; first tarsal segment of hind legs only slightly longer than second ..... *F. armaticrus*, K.†
   Femora and tibiae dark brown, tarsi yellowish ; first tarsal segment of hind legs decidedly longer than second ..... *F. auripes*, sp.n.

4. Wings adorned‡..... 5
   Wings unadorned ..... 9

5. Wings with a single pale spot, situated about the middle of the anterior border, in the region of the end of the costa ..... 6
   Wings with several pale spots ..... 7

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* In all the species examined by us [excepting *F. ingrami*] the ratio of the length of the first tarsal segment of the hind legs to the second, although varying somewhat, is similar in the two sexes, and in this key where only one sex is known we have assumed that the ratio is approximately the same in both sexes.

† It is dificult to place this species in the key correctly, because part of the description appears to have been dropped out by the printer ; we have, therefore, inserted it twice.

‡ By adornment we mean with pale spots. No account is taken, however, of the usual small bare area just beyond the end of the costa which may simulate a spot, nor of appearances due to the darkening of the anterior part of the wing produced by greater density of hairs and scales in this region, nor of the pallor sometimes visible at the extreme base of the wing.

6. Hind femora with a dark brown apical band, and hind tibiae with a dark brown basal band ..... *F. biannulata*, sp.n.
Hind femora with a narrow sepia-brown band or spot a little distal to the middle ..... *F. castanea*, (Walk.)
Hind femora without bands or spots..... *F. castanea* (Walk.) var. *incomptifeminibus* (Aust.)

7. Antenna of female with last segment twice as long as broad ; that of the male with 13th and 14th segments sub-equal, about half the length of the 12th..... *F. chrysolopha* (K.)
Antenna of female with last segment about three or four times as long as broad ; that of the male with 13th segment longer than the 14th and more than half the length of the 12th ..... 8

8. Harpes of male large, hook-like ; antenna of female with segments 4 to 10 flask-shaped, about two to two-and-a-half times as long as broad, bearing long, slender, pointed spines ..... *F. squamipennis*, sp.n.
Harpes of male long, stout, with rather blunt ends ; antenna of female with segments 4 to 10 less than twice as long as broad, bearing very stout, blunt spines ..... *F. lepidota*, sp.n.
Harpes of male long, with filiform ends ; antenna of female as in *F. lepidota* ..... *F. venusta*, sp.n.
Harpes of male long, with ends a little expanded like a duck's head ; female unknown..... *F. pampoikila*, sp.n.

9. Hind femora with dark bands or other markings 10
Hind femora without dark bands or other markings .....13

10. Hind tibiae almost entirely dark brown, only the extreme apex being slightly tawny ..... *F. nigrotibialis*, sp.n.
Hind tibiae with at most a single dark brown band... *F. inornatipennis* (Aust.) var. *ornaticrus*, var.n.
Hind tibiae with two dark brown bands .....11

11. Bands on the hind tibiae at the extremities, with a clear ring between them ..... *F. nilotica* (K.)
Distal dark band on the hind tibiae not reaching the extremity of the segment .....12

12. Darkish brown ; very hairy ; antenna of male with the combined length of segments 3 to 11 almost equal to that of segments 12 to 15, segment 12 three-and-a-half times as long as segment 11 ... *F. hirsuta*, sp.n.
Yellow or whitish ; antenna of male with combined length of segments 3 to 11 as long as that of segments 12 to 15, segment 12 two-and-a-half times as long as segment 11..... *F. ornatipes* (K.)
Light brown ; antenna of male with combined length of segments 3 to 11 greater than that of 12 to 15, segment 12 only twice the length of segment 11..... *F. tigripes*, sp.n.

13. First tarsal segment of hind legs slightly longer than second..... *F. armaticrus*, K.
First tarsal segment of hind legs clearly shorter than second, one-half to two-thirds the length 14

14. Legs yellowish ; terminal segments (11 to 15) of antenna of female over four times as long as broad ; spermathecae oval, 80-130 $\mu$ by 60-80 $\mu$, practically no part of the duct chitinised ..... *F. inornatipennis* (Aust.)
Legs brown ; terminal segments of antenna of female about three times as long as broad ; spermathecae smaller, subspherical, diameter about 40 $\mu$, the commencement of the duct chitinised for a short distance, about 7 $\mu$ ..... *F. nilotheres*, M.

15. First tarsal segment of hind legs longer than the second.....16
First tarsal segment of hind legs shorter than the second, or of about equal length.....30

16. Wings adorned .....17
Wings unadorned .....18

17. Wings with two pale, yellowish, elongated spots on the anterior border, one covering the distal end of the third vein, and one between the anterior border of the wing and the fork of the intercalary vein ; first and third veins forming a small distal cell ..... *F. tangae*, K.
Wings with grey markings and two dark spots on the anterior border ; first and third veins completely fused ..... *F. puncticollis* (Becker)

18. Eyes hairy ..... *F. aethiopiae*, sp.n.
Eyes bare.....19

19. Antenna of female with the last five segments elongated, an abrupt change of shape between the tenth and eleventh segments .....20
Antenna of female with flagellum segments forming an almost continuous series, no abrupt change of shape between the tenth and eleventh segments .....25

20. First tarsal segment of hind legs only slightly longer than the second, less than twice as long.....21
First tarsal segment of hind legs twice the length of the second or longer.....23

21. First and third veins of the wing joined for their whole length ..... *F. aplanota* (K.)
First and third veins forming a distinct distal cell 22

22. Reddish, with pale yellow legs (*i.e.*, as in *F. aplanota*) ; first and third veins of wing fused in proximal half ; scutellum brown (*i.e.*, as in *F. aplanota* female) ..... *F. seychelleana* (K.)
As *F. seychelleana* but thorax orange-red and abdomen very dark brown..... *F. seychelleana* var. *fulvithorax* (K.)

Reddish, with pale yellow legs; first and third veins of the wings fused in proximal two-thirds; scutellum yellow in female, dark brown in male... *F. rufescens* (K.)

Usually dark brown, but sometimes paler brown, with yellowish-brown legs; first and third veins of wing in female with proximal halves contiguous, but actually forming a very narrow proximal cell difficult to distinguish; scutellum brown in both sexes ..... *F. ingrami*, ♀, Carter *

Yellowish-white or reddish, with yellow legs; first and third veins of wing confluent in their proximal halves; scutellum (female) brown or dark brown ..... *F. kribiensis* (K.)

23. Terminal segments of antenna of female three to four times as long as broad; very dark brown species with yellowish legs; bifurcation of the fifth vein of wing proximal to the end of the costa..... *F. mahensis* (K.)

Terminal segments of antenna of female twice as long as broad .....24

24. Very dark brown, legs brownish ..... *F. falcinella* (K.)

Yellow, legs whitish ..... *F. sulfurea*, K.

25. First tarsal segment of hind legs about one-and-a-half times as long as the second .....26

First tarsal segment of hind legs over twice the length of the second .....28

26. Dorsum of thorax with three broad, brown, longitudinal stripes ..... *F. abyssiniae* (K.)

Dorsum of thorax uniformly coloured .....27

27. First and third veins of the wing united ..... *F. tavetae*, K.

First and third veins of the wing forming a distal cell ..... *F. egypti*, M.

28. Dorsum of thorax with three broad, darkish brown, longitudinal stripes ..... *F. exigua*, sp.n.

Dorsum of thorax uniformly coloured .....29

29. Antenna of female with combined length of segments 3 to 10 one-quarter longer than that of segments 11 to 15; whorls of 6 or 7 hairs ..... *F. seneveti*, K.

Antenna of female with combined length of segments 3 to 10 equal to that of segments 11 to 15; whorls of 10 to 12 hairs ..... *F. seneveti* var. *biskraensis*, K.

30. Wings adorned .....31

Wings unadorned .....36

31. Wings with several pale spots ..... *F. pretoriana* (K.)

Wings with a single pale spot about the middle of the anterior border, in the region of the end of the costa .....32

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* In the male *F. ingrami*, the first tarsal segment of the hind legs is shorter than the second.

32. Legs with darker brown bands or spots .....33
Legs without such markings .....34

33. Hind femora with a narrow sepia-brown band or spot a little distal to the middle, hind tibiae unadorned; harpes of the male with pointed but not filiform ends..... *F. castanea* (Walk.)*
Hind femora with a dark brown apical band, hind tibiae with a dark brown basal band; harpes of male with filiform ends, but no patch of teeth at the base of the side-pieces..... *F. biannulata*, sp.n.*
Hind femora with an inconspicuous spot at the distal end, hind tibiae sometimes with a trace of a band a little below the knee; harpes of male with filiform ends, side-pieces with a patch of spines or teeth on the inner basal aspect ..... *F. ashantii*, sp.n.

34. Whitish; combined length of segments 3 to 10 of antenna of female a half greater than that of segments 11 to 15 ..... *F. leucochaeta* (K.)
Brown or dark brown; combined length of segments 3 to 10 of antenna of female only slightly greater than that of segments 11 to 15...35

35. Last five segments of the antenna of the female elongated, segments 11 to 14 two to three times as long as broad; an abrupt change of shape between segments 10 and 11 ..... *F. castanea* (Walk.) var. *incomptifeminibus* (Aust.)*
Last five segments of antenna of female not so much elongated, segments 11 to 14 nearly twice as long as broad; change of shape between segments 10 and 11 not abrupt..... *F. striaticornis* (K.)

36. Legs with darker brown bands or spots.....37
Legs unadorned with darker markings .....41

37. Hind legs with a dark spot at the distal end of the femora only.....38
Hind legs with bands on both femora and tibiae.....39

38. Thorax dark brown; terminal segments of the antenna of the female three times as long as broad ..... *F. lasionota* (K.)
Thorax pale yellow; terminal segments of the antenna of the female only twice as long as broad ..... *F. lasionota* var. *callithorax* (K.)

39. Yellow; thorax with three broad, brown, longitudinal bands on the scutum..... *F. fusciforceps* (K.)
Mainly dark brown, with yellow legs; thorax without dark longitudinal bands on the scutum .....40

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* *F. castanea*, *F. castanea* var. *incomptifeminibus* and *F. biannulata* are inserted in the key twice as the scales are apt to be overlooked.

40. Hind legs with distal third of femora and proximal third of tibiae brown ..... *F. kilemae*, K.
Hind legs with femora except the base, and tibiae except the extremity, brown ..... *F. sahariensis*, K.

41. Legs dark brown .....42
Legs yellowish .....43

42. Very dark brown; dorsum of thorax showing indistinctly three broad longitudinal stripes ..... *F. melanchroa*, sp.n.
Dull darkish brown; dorsum of thorax uniformly darkish brown ..... *F. nigeriensis*, sp.n.

43. Females known .....44
Only males known .....45

44. Terminal segments of the antenna of the female elongated, clearly differentiated from the basal segments..... *F. maura* (K.)
Terminal segments of the antenna of the female not clearly differentiated from the basal segments; no abrupt change of shape between segments 10 and 11..... *F. radiifer* (K.)

45. First and third veins of the wing confluent, no cell ..... *F. niligena* (K.)
First and third veins forming a distal radial cell...46

46. Very dark brown, legs pale yellow; hind tarsi with the first segment less than half the length of the second..... *F. psilonota* (K.)
Not so dark brown; hind tarsi with the first segment only slightly shorter than the second 47

47. Harpes without rod-like posterior structures ..... *F. ingrami*, ♂, Carter
Harpes with rod-like posterior processes which terminate in sharp points ..... *F. catanei*, K.

### *FORCIPOMYIA CASTANEA* (Walk.)

Length of body* (average), 2 mm.; length of wing, 1·4 mm.; greatest breadth of wing, 0·5 mm. The size is variable, the length of the body of the females measured by us ranging from 1·4 mm. to 2·2 mm. In the male the length of the body is greater than in the female, owing to the size of the hypopygium, and the wings are narrower.

*Head* dark sepia-brown; occiput bearing numerous, long, dark brown hairs which overhang the eyes. Eyes bare†; broadly

* In all cases this measurement is taken from the anterior margin of the thorax to the tip of the abdomen of specimens mounted in pure carbolic acid.

† The cuticle between the eyes and the bases of the antennae is covered with minute hairs, and a very few similar hairs may be found occasionally between the facets at the extreme inner margins of the eyes. No account is taken of such hairs, however, in the description of this insect, or of the following species.

contiguous above in both sexes, but with the facets separated by a narrow line. Clypeus dark brown; in the female somewhat elongated, bluntly rounded distally, bearing about sixteen dark brown hairs which are mostly grouped along the middle line; in the male shorter, more rounded. Proboscis dark brown, in the female rather longer than the head, in the male shorter. Labium shorter and broader than in *Culicoides*. Stylets well-developed, chitinous. Labrum in the female highly chitinised, with sides parallel to the distal fourth, then gradually tapering to a narrowly rounded apex and bearing a delicate fringe; in the male similar but less highly chitinised and more sharply attenuated distally, with dense hair-like processes over the distal third. Mandibles in the female long, broad, strongly chitinised, blade-like and pointed, with numerous (about twenty-five) closely apposed, blunt teeth extending almost half the length of the inner margin; in the male similarly shaped, well-developed, highly chitinised but not so strongly as in the female, without teeth. Maxillae very similar to those of *Culicoides*, extending forwards rather less than the mandibles, less highly chitinised, more delicate and narrower, the distal fourth with about ten delicate, rather widely spaced teeth; in the male well-developed, thinly chitinised, apex with fairly long and strong hairs. Hypopharynx in the female highly chitinised, similarly shaped to that of *Culicoides*, but distally somewhat narrower, broad at the base, tapering gradually to the distal fourth and then more rapidly to the pointed apex, distal margin smooth; in the male as in the female, dense, sharply pointed. Palpi (fig. 1, A and B) dark brown, bearing rather numerous and long dark brown hairs: first segment small, second, fourth, and fifth small, sub-equal, third large, longer than any two of the other segments together, basal half swollen and furnished with a large, oval sensory pit with a circular orifice. *Antennae* uniformly darkish brown, bearing similarly coloured hairs. In the female segments 3 to 10 bearing long, stout spines which are almost straight, colourless, and about as long as the segments, and whorls of about twelve dark brown hairs which are about twice as long as the segments; segments 11 to 15 bearing numerous smaller spines and small basal whorls of hairs. First segment a ring of dark brown chitin, bearing numerous dark brown hairs. Torus dark brown, sub-spherical, bearing a few dark brown

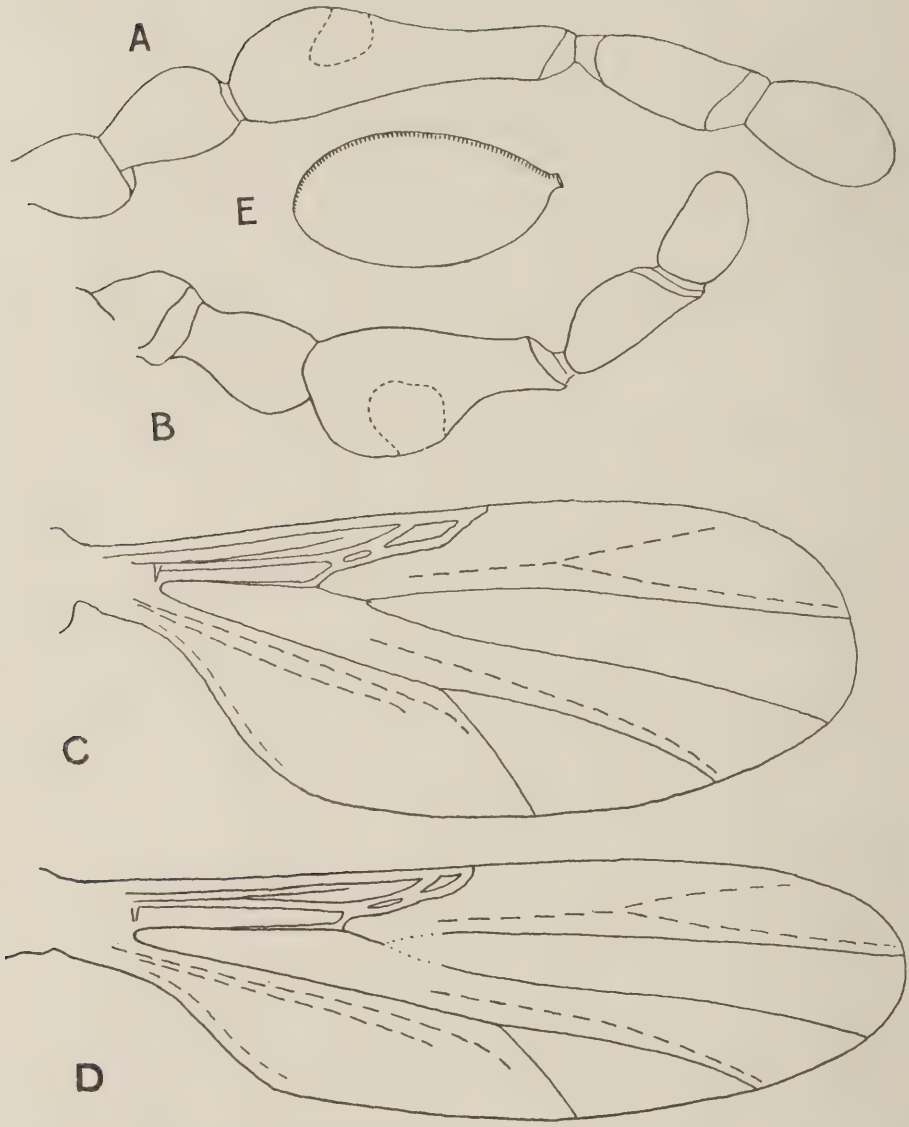


FIG. 1. *Forcipomyia castanea* (Walk.). *A*—Palp of ♂. *B*—Palp of ♀. *C*—Venation of wing, ♀. *D*—Venation of wing, ♂. *E*—Spermatheca. *C* and *D* × *c.* 80; others × *c.* 400.

hairs. Third segment sub-spherical, with a short stalk. Segments 4 to 10 sub-equal, a little longer than broad (11 to 7 units) somewhat flask-shaped. Segments 11 to 15 elongated ; 11 to 14 sub-cylindrical, contracted into a neck just before the apex, sub-equal, about two to three times as long as broad ; the fifteenth stouter, slightly longer, ending in a blunt, club-shaped stylet. The combined length of segments 11 to 15 about equal to that of segments 3 to 10 and a little greater than that of segments 4 to 10, namely, averaging in three specimens approximately 110, 100, and 90 units respectively. In the male segments 3 to 11 bearing spines, those on the basal segments similar to those of the female, but the others smaller ; and large whorls of brown hairs which on the basal segments are almost transverse and situated about the middle of the segments, but become more oblique towards the eleventh segment. The whorls of hairs together form a dense, dark brown plume which reaches nearly to the level of the extremity of the antenna. Torus dark brown, very large, without hairs. Third segment sub-spherical, with a rather long stalk. Segments 4 to 11 sub-spherical to irregularly ovate, becoming narrower and longer towards the eleventh. Segments 12 to 15 elongated, segments 12 to 14 slightly expanded basally and measuring respectively in length and basal breadth about 47 by 8, 28 by 7, and 22 by 5 units ; the fifteenth segment broader, about 32 by 8 units, and ending in a club-shaped stylet. The combined length of segments 12 to 15 slightly greater than that of segments 3 to 10, *e.g.* about 130 to 115 units. *Thorax :* dorsum uniformly dark sepia-brown, but sometimes showing traces of the narrow, pale, admedian lines which in other species are distinct and divide the scutum into three broad longitudinal stripes ; clothed with short, dark brown hairs, intermixed with which are some paler brown hairs. Pleura pale yellowish-brown or cream-buff coloured, sometimes with a dark brown spot beneath the wings. Scutellum dark sepia-brown, bearing in both sexes very numerous long dark brown setae and hairs. Post-scutellum dark sepia-brown, without a central depression. *Wings* (fig. 1, C and D) entirely covered with minute microtrichia and densely clothed with dark brown hairs which are darker and denser on the anterior margin and at the tip of the wing than on the other parts, and some of which are distinctly scale-like ; the extreme base of the wing is pale, buff-coloured, and

there is a small pale, yellow or golden spot about the middle of the anterior border which just covers the junction of the third vein with the costa. Fringe well-developed. In the male the wings are much paler than in the female and the pale spot is indistinct; indeed the area distal to it, which is covered with dark hairs, is often more conspicuous; the wings are also narrower and more translucent. Veins yellowish. Costa reaching to about the middle of the wing. First and third veins forming two cells, the distal one the larger, distinct in both sexes, the proximal one slit-like, barely perceptible in the male. Petiole of the fourth vein longer than the cross-vein. Bifurcation of the fifth vein distal to the bifurcation of the fourth, and in the female at about the level of the end of the costa, but in the male some distance distal to this level. Intercalary veins well-developed, that near the tip of the wing with a long stem. Halteres with creamy-white knobs. *Legs* pale yellowish-brown, clothed with hairs and scales of the same colour which are longer in the male than in the female. Hind femora usually with a narrow sepia-brown band a little distal to the middle; this band varies in size and distinctness to a considerable degree, is sometimes reduced to a mere spot, or may even be entirely absent. *F. incomptifeminibus* (Aust.) must therefore be regarded as only a variety of *F. castanea*. Hind coxae with a dark brown spot on the posterior aspect. Terminal tarsal segments usually slightly infuscated. Femora unarmed. Tibiae with lanceolate scales; fore tibiae with a stout pale yellow apical spur. In both sexes the first tarsal segment slightly shorter than the second on all the legs; the proportions of the hind tarsal segments about 44 : 67 : 41 : 28 : 24. Claws of all the legs equal, small, rather less than half the length of the fifth tarsal segment, curved but not so much as to form a semicircle, and simple in the female, but in the male longer and more slender, more strongly curved than in the female, and with bifid ends. Empodium nearly as long as the claws, with long hairs on both lateral margins. *Abdomen* in the female short and broad, dorsum dark sepia-brown; sides, venter, and cerci paler, yellowish, rather densely clothed with hairs and a few scales which are mostly dark brown; in the male slender, mainly yellowish, but with sepia-brown bands about the middles of the segments which are very variable, but are usually broader on the last three segments, rather sparsely clothed with hairs,

and with a large sepia-brown hypopygium. Spermathecae (fig. 1, E) two, dark brown, highly chitinised, oval or egg-shaped, length about 90$\mu$, greatest breadth about 50$\mu$; the duct narrow, chitinised for only a short distance (about 7$\mu$) at its commencement.

Hypopygium (fig. 2). Ninth segment: tergite short and broad, somewhat 'lozenge' shaped, densely clothed with long hairs, posterior margin rounded, with protruding backwards beyond it, a membranous extension bearing on each side a long hair and a well-developed, slightly chitinised process, furnished with two long and two short hairs; sternite rather broad, not excavated in the middle line posteriorly, covered by long hairs. Forceps: side-pieces large,

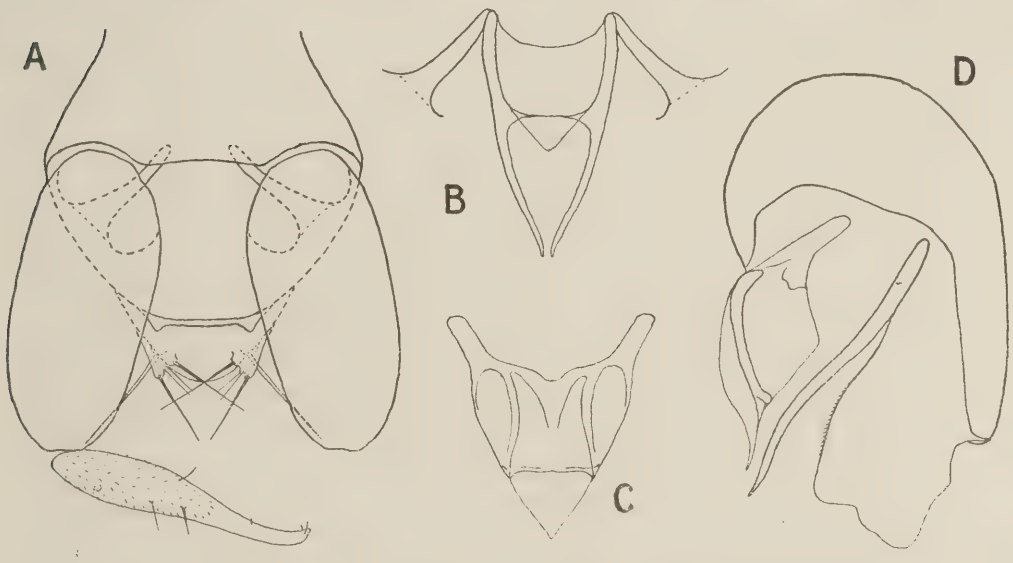


Fig. 2. *Forcipomyia castanea* (Walk.). ♂: Diagrams of hypopygium. *A*—Ninth segment and forceps, ventral view. *B*—Harpes, ventral view. *C*—Aedoeagus, ventral view. *D*—Lateral view to show the relative positions of harpes and aedoeagus.

twice as long as broad, straight, densely clothed with hairs some of which are very long, prolonged at its proximal end dorsally as a long rod-like process which articulates with the harpe; claspers similar in form to those of *Culicoides*, about four-fifths the length of the side-pieces, highly chitinised at the distal end, pubescent over the basal half and bearing a few larger hairs. Harpes long, almost straight, rods directed backwards and a little ventrally, moderately well-chitinised, tapering at the apex to sharply pointed tips, their

basal halves united by a thin sheet of chitin which is prolonged posteriorly and medially as a triangular process. Aedoeagus short and very broad, the greater part of it thinly chitinised, terminating posteriorly in a triangular membranous process.

The early stages of *F. castanea* were found by us in 1920 at Accra and Nsawam in rotting fibrous matter at the bases of banana stumps, and were also procured by isolating gravid females taken on the windows of the laboratory in tubes containing rotting vegetable matter. In one such tube in which a single female had been imprisoned on the 15th November, 1920, larvae were first seen on the 23rd (at which time they were already about 2 mm. long), pupae on the 28th, and adults of the new generation on the 30th. From this tube were obtained three adults, fifteen pupae, and thirteen larvae ; the original female had therefore laid thirty-one eggs at least. At this time a full description of the larva and pupa was prepared but was not published, and now, as Saunders (1924) has recently given us a most able and detailed study of the anatomy of the early stages of *Forcipomyia* it is possible to curtail it and in some respects modify it.

PUPA (fig. 3, A to E). The duration of the pupal period is about two days. The larvae pupate on the surface of the medium in which they are living, and the pupae are at first a creamy-white colour but rapidly darken. If left undisturbed, the pupae do not change position appreciably before hatching ; if immersed in water, however, they move their abdomens actively in a vigorous but rather ineffective attempt to rescue themselves. Length about 3·5 mm., greatest breadth about 0·7 mm., well-chitinised, the posterior half of the abdomen loosely enveloped by the larval pelt. The integument is shagreened and bears numerous small tubercles covered with coarse squamose spines, and sometimes armed with short spines or bristles.

*Cephalo-thorax* relatively large and broad, extended posteriorly over the middle of the first abdominal segment in the form of a relatively long and narrow process. Respiratory trumpets rather short (about 260$\mu$), with a short broad stem and a large expanded distal extremity. The main tracheal trunk is wide, straight in the proximal part of the trumpet, and curved like a hook in the expanded distal part ; at the point where the trumpet begins to expand is

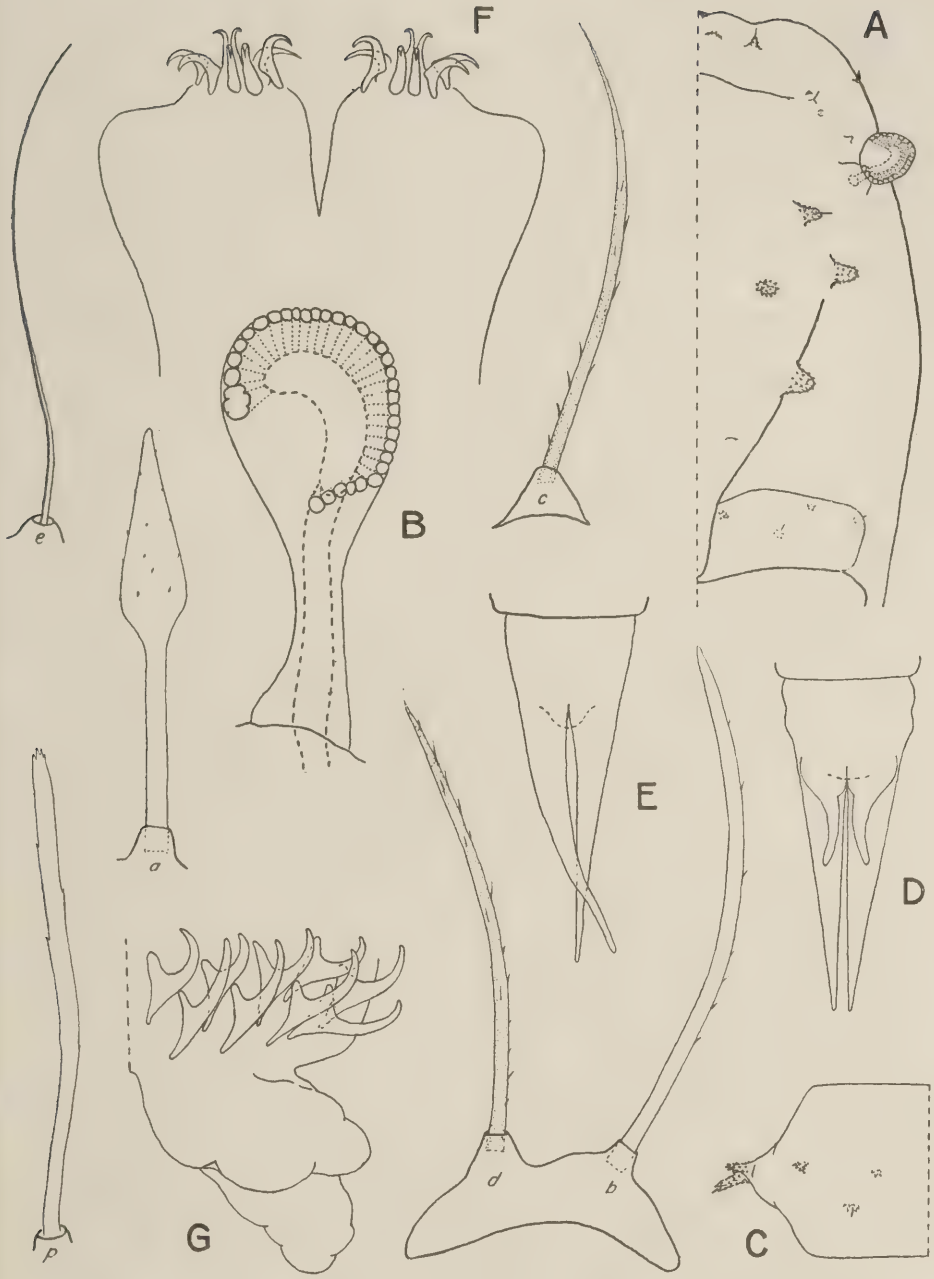


FIG. 3. *Forcipomyia castanea* (Walk.). Pupa: *A*—One half of the dorsal part of the cephalothorax showing the chief tubercles, *B*—Respiratory trumpet, *C*—One half of the fourth abdominal segment, dorsal view, showing the tubercles, *D*—Posterior extremity, ♂, dorsal view, *E*—Posterior extremity, ♀, dorsal view. Larva: *F*—Prothoracic pseudopod, ventral view; *G*—Anal blood-gill and hooks on one half of the posterior extremity of the body, ventral view; *p*, *a*, *b*, *c*, *d*, and *e*—macrochaetae lettered as by Saunders. *A*, *C*, *D*, and *E*, × *c.* 80; others × *c.* 265.

a small posterior diverticulum. From the whole length of the external border of the tracheal trunk in the distal expansion arises a fan-like arrangement of short branches (the number varying greatly, certainly from twenty-three to fifty-four), whose distal extremities form two-thirds of a circle (open only at the proximal side) which is bridged by a delicate granular membrane. The wing and leg cases are attached to the sides and extend backwards on the ventral aspect as far as the anterior border of the third abdominal segment. There are a few tubercles on the cephalo-thorax, all of them rounded or conical prominences, and most of them covered by squamose spines. The following may be distinguished. Situated on the operculum are three low, rounded tubercles which are unarmed; one, the smallest, in the middle line a little behind the anterior margin of the cephalo-thorax, and two, one on each side, at the anterior margin. The latter possibly correspond with the anterior marginal tubercles of *Culicoides*. Anterior dorsal: a small tubercle bearing a short spine, situated a little behind and external to the anterior marginal tubercle. Anterior dorso-lateral: a small tubercle, unarmed, situated just in front of and external to the base of the respiratory trumpet. A little anterior and internal to this tubercle are three other small tubercles arranged in an antero-posterior row, the middle one bearing a short spine and the other two unarmed. Laterally or ventro-laterally, a little posterior to the respiratory trumpet, are two or three small elevations which are unarmed. Dorsal tubercles: situated on each side of the central portion of the thorax are four relatively large, conical, but not finger-like tubercles arranged in the form of a diamond; the anterior one bears a short spine, the other three are unarmed. There are traces of other unarmed tubercles rather more posteriorly, just anterior to the first abdominal segment, but they are very indistinct. No tubercles could be distinguished on the ventral aspect of the cephalo-thorax; the ventro-median is represented by a minute hair.

*Abdomen* directed straight backwards and partly enclosed in the larval pelt. It consists of nine well-developed segments, and is broad at the base, narrowing gradually but rather rapidly towards the apex. The first segment is short and narrow, the second deep and broad, the ninth elongated, and the intermediate segments rather broad and short, and narrowing successively as the posterior

extremity is approached. The last segment terminates in two long, straight, nearly parallel, lateral processes which taper to pointed extremities; in the male this segment is furnished also with two shorter, finger-like, dorsal processes. The abdominal tubercles are covered with squamose spines and are mostly small; on a typical segment, for example the fourth, they are arranged as follows on each side. On the dorsal surface are three tubercles, two admedian and one dorso-lateral; the admedian tubercles are small, the anterior one unarmed, the posterior one, which is situated rather more externally, bearing a small spine; the dorso-lateral tubercle is small and unarmed, it lies at about the same level as the anterior admedian tubercle. At the side are two relatively large, prominent, finger-like tubercles, the one dorsal to the other, situated about the middle of the segment, and bearing minute spines; the more ventral of the two is the larger. On the ventral surface only a small double tubercle appears to be present, this is situated about the middle of the segment and bears two small spines. The size of the abdominal tubercles diminishes as the posterior end is approached; they may be almost obsolete and represented only by one or two squamose spines on the distal segments. The arrangement of the dorsal tubercles described above applies only to segments 2 to 5, on segments 6 to 8 the tubercles are almost completely absent, being replaced by a few socket-like marks and two or three small spines one of which, situated in a submedian position, is rather strongly developed. The ventral tubercles are recognisable as such on segments 3 to 5 only, on segments 6 to 8 they are represented by two minute spines. On the first segment there are four small tubercles, and two socket-like marks; on the last segment no definite tubercles are discernible, but laterally are three insignificant elevations.

Larva (fig. 3). The body of the young larva has a dull white colour and is semi-transparent; when more mature it is darker, chiefly owing to the dark brown intestinal contents showing through the cuticle. The head is dark brown. The larva is fairly active and browses on the surface of damp vegetable débris. In this situation it is not easily seen, unless the light catches the tips of the dorsal spines and shows them as a long double row of pale, glistening points. In progression the head is used freely. The anal blood-gills are not easily seen in the living larva and are retracted in dead specimens

unless they have been killed and fixed by hot (65° C.) solutions. When about to pupate, the larva ceases to browse, settles in some chosen spot on the surface of the medium, and becomes whiter and more opaque ; other changes that may be noted are a thickening of the three anterior body segments due to the development of the imaginal discs, and a retraction of the eyes. The duration of the larval period is about ten days.

Length when mature, 4 mm. to 4·5 mm. *Head* strongly chitinised, almost black anteriorly, showing a colour pattern somewhat similar to that of *F. picea* in Saunders' figure. Antennae of the usual form, short (about 90$\mu$) and straight, and bearing at the end a short, pointed process. The paired sensory hairs *p.* and *q.* of Saunders are long, stout, slightly curved, feebly chitinised, somewhat similar to those of *F. picea* but slightly hairy or serrated. The other hairs of the head apparently similar to those of *F. bipunctata.* Mouth parts similar to those of *F. bipunctata* ; mandibles with three large teeth at the distal end.

*Body* with cuticle covered with spicules. Armature of bristles and spines on the abdominal segments as follows. A pair of long spear-shaped admedian dorsal spines (macrochaeta *a* of Saunders) arising from tubercles which in mature larvae are connected together across the back by a narrow chitinous band ; two pairs of long, slightly curved, dorso-lateral setae (*b* and *d*) arising from large double tubercles ; a pair of setae (*c*) similar to *d* but longer, arising from conical tubercles ; and two pairs of long, stout, simple, ventro-lateral hairs (*e* and *f*) arising from small tubercles. The spear-shaped dorsal spines are about 200$\mu$ long, the blade-like distal portion measures about 25$\mu$ at its broadest part, has a long point, and is sparsely covered with spicules ; the length of the shaft is only slightly greater than that of the blade-like portion. The description of these spines as spear-like is scarcely appropriate, as the distal portion is not flat but is cone-shaped ; in lateral view, however, they appear spear-like, and as this descriptive term has been used before, it is again used here. There are twelve pairs of these spines, the first pair being rather small, and the last modified, the tubercles from which they arise being broadly fused and the distal ends of the spines being only slightly broader than the shafts. The two dorso-lateral setae (*b* and *d*) arise the one slightly more dorsally than the

other; the former (*b*) is not highly chitinised, is very sparsely clothed with barbs, is rather blunt-ended, and is similar to the head hairs *p* and *q*; the latter (*d*) is shorter, highly chitinised, dark coloured, well-barbed, and more pointed.

The prothoracic pseudopod is cleft distally but not very deeply, the two parts not widely separated, broad at their extremities, with the crowns of hooks (six strong, and two more delicate) situated on their inner sides. Anal blood-gills bilobed. Posterior pseudopod armed with, usually, nine large hooks in two rows on each side of the middle line. Malpighian tubules apparently three.

Egg. The eggs are a dull white colour, smooth, ellipsoidal or whetstone-shaped; length about 205$\mu$, middle breadth about 78$\mu$. When laid they apparently adhere by their sides; they may sometimes be seen thus, forming a chain, whilst still attached to the body of the female.

Gold Coast: Accra, numerous specimens, both males and females, taken on the windows of the laboratory in the evening at all times of the year. Numerous specimens also reared from eggs laid by females in captivity, and hatched from rotting banana fibre. Nsawam, reared from vegetable matter from a rot-hole in a tree.

### *FORCIPOMYIA BIANNULATA*, sp.n.

Length of body (average), 2 mm.; length of wing, 1·3 mm.; greatest breadth of wing, 0·3 to 0·5 mm. In the male the wings are relatively longer and narrower than in the female. With narrow scales.

*Head* dark brown. Eyes bare, broadly contiguous above in both sexes, the facets separated by a narrow line. Clypeus, proboscis, and palpi dark brown. Palpi similar to those of *Forcipomyia castanea*. *Antennae* brown. In the female segments 3 to 10 bearing stout, curved spines, about as long as the segments, colourless, with rather blunt ends; and whorls of about seventeen hairs. The flagellum segments sub-equal, forming an almost continuous series, the fifteenth, however, slightly longer than the rest; segments 3 to 10 somewhat flask-shaped, segments 11 to 14 with a distinct 'collar,' and the last segment ending in a small blunt stylet. The combined length of segments 11 to 15 less than that of segments 3 to 10,

or 4 to 10, *e.g.* in one specimen measuring 77 units as compared with 118, or 101 units. In the male similar to those of *F. castanea*, but the last segment perhaps a little shorter, the lengths of the five terminal segments in one male being 12, 47, 26, 20, and 25 units respectively. The combined length of segments 12 to 15 equal to that of segments 3 to 11. *Thorax* uniformly dark brown dorsally, densely clothed with yellowish hairs. Pleura paler, golden-brown, paler in the male than in the female, with a darker brown spot immediately under the base of the wing. Scutellum and post-scutellum dark brown, the former bearing numerous long, yellowish bristles and shorter hairs which are more abundant in the female than in the male. *Wings* similar to those of *F. castanea*, densely clothed with dark brown hairs and narrow scales, and with a small pale, yellowish spot at the distal end of the costa enveloping about two-thirds of the distal radial cell. The wings in the male are narrower than in the female, less hairy, but with a distinct pale spot, and some infuscation in the neighbourhood of the intercalary vein and of the rami of the fourth and fifth veins. Venation similar to that in *F. castanea ;* but the first and third veins are fused basally so that the first radial cell is obsolete ; the second radial cell, however, is well-formed. Halteres with cream-coloured knobs and almost colourless stems. *Legs* yellowish-brown, terminal tarsal segments somewhat infuscated. Hind femora with a broad dark brown apical band which completely encircles the limb, hind tibiae with a similar band covering the basal third, knees not infuscated. The development of the bands on the hind legs is subject to some degree of variation. Legs moderately hairy and bearing also narrow scales, a few of the hairs, such as two on the ventral aspect of the first tarsal segment, being exceptionally long. The middle and hind tibiae of the female bear also one or two peculiar short, hastate hairs or spines (fig. 4, D) : these spines are not present in the male. In the male the first tarsal segment about equal to the second on the fore legs, but shorter (about three-quarters the length) on the middle and hind legs ; in the female the first tarsal segment about equal to the second on the middle and hind legs, longer (about one-third longer) on the fore legs. Claws and empodium similar to those of *F. castanea*. *Abdomen* in the female dark brown dorsally, paler brown ventrally and at the sides, clothed with dark and light brown

hairs and narrow scales ; in the male paler, light brown, with broad dark brown bands on the segments which are broadest ventrally, and larger and more complete towards the posterior extremity ; clothed with rather sparse and long hairs. Hypopygium dark brown. Spermathecae two, dark brown, highly chitinised, more or less oval, often unequal (in one extreme example measuring 68$\mu$ by 65$\mu$ and 133$\mu$ by 87$\mu$ respectively) ; the commencement of the duct chitinised for only a short distance (about 4$\mu$).

Hypopygium (fig. 4, A to C) similar to that of *F. castanea* but more highly chitinised and more deeply infuscated. Ninth segment similar to that of *F. castanea*, but sternite less hairy. Forceps similar to those of *F. castanea*, but darker, the tips of the claspers being dark brown ; side-pieces clothed with strong hairs which, however, are not very long. Harpes long, slender rods tapering to

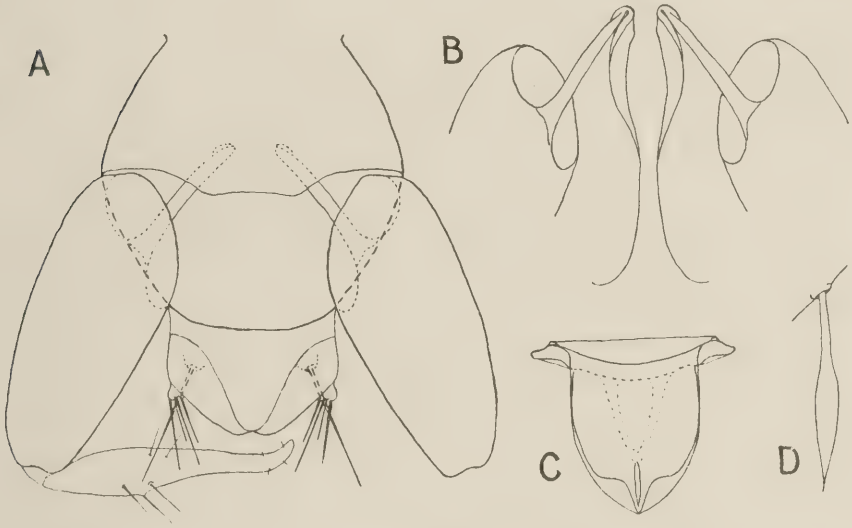


Fig. 4. *Forcipomyia biannulata*, sp.n. ♂ : *A*—Ninth segment and forceps, *B*—Harpes, *C*—Aedoeagus, all ventral views. ♀ : *D*—Hastate tibial hair.

filiform extremities, similar to those of *F. ashantii*. Aedoeagus broad and shield-like in ventral view, somewhat resembling that of *F. castanea* but differing in detail and more highly chitinised.

Gold Coast : Accra, numerous specimens, both males and females, taken in the evenings on the windows of the laboratory at all times of the year, but more abundantly during the second half.

NIGERIA: Afikpo, 2.6.1910, 1 ♀ (Dr. J. J. Simpson); Lagos, June, 1921, 1 ♀ (Dr. H. A. Foy).

NYASALAND: South-east shore of Lake Nyasa, March, 1910, 1 ♂ (Dr. S. A. Neave).

This species resembles *F. castanea* but may be readily distinguished from it by the presence of a dark brown basal band on the tibiae of the hind legs.

### *FORCIPOMYIA AURIPES*, sp.n.

Length of body (one male), 2·9 mm.; length of wing, 1·8 mm.; greatest breadth of wing, 0·4 mm. A large, dark brown species with dark brown femora and tibiae and yellowish tarsi.

*Head* dark brown. Eyes bare; broadly contiguous above, but actually very narrowly separated. Clypeus and proboscis brown. First and second segments of the palp brown, other segments missing. *Antennae* incomplete in the single specimen examined. Basal segments of the flagellum brown apically, yellowish basally, the line of insertion of the whorl hairs separating the two colour zones; plume dark brown with a paler brown tip. The length and greatest breadth of the eleventh and twelfth segments about 20 by 9, and 78 by 8 units respectively. Segments 13 to 15 missing. *Thorax* almost uniformly dark brown, but showing traces of the three longitudinal stripes on the dorsum so common in the genus; pleura slightly paler brown than the dorsum. Hairs rather sparse, no scales. Scutellum and post-scutellum dark brown; the former bearing about twenty bristles and hairs. *Wings* long and narrow, pale brown, closely covered with minute microtrichia of the usual form and densely clothed with decumbent hairs; bearing also on the costa and first and third veins long lanceolate scales. No bare areas along the veins. Costa reaching beyond the middle of the wing (65 : 101 units), to the distal third. First and third veins contiguous basally, the proximal cell obsolete or a mere slit, the distal one long and narrow. Bifurcation of the fifth vein proximal to the end of the costa, at about the level of the base of the distal radial cell; anterior ramus reaching the margin of the wing a considerable distance beyond the level of the end of the costa. Hairs of fringe with rather long fimbriae, sub-plumose. Halteres darkish

brown, knobs containing a white pigment. *Legs* with dark brown femora and tibiae, yellowish tarsi. Scales present on the femora and distal segments. First tarsal segments two-and-a-half, two, and one-and-a-half times as long as the second on the fore, middle, and hind legs respectively. Claws about half the length of the fifth tarsal segment; empodium as long as the claws, hairy. *Abdomen* dark brown, especially posteriorly, and in the single specimen examined containing much actually black pigment. Hairs on the posterior part of the abdomen long, especially those on the ventral surface. Scales present on the dorsal surface.

HYPOPYGIUM (fig. 5). Ninth segment very dark brown, both tergite and sternite well-clothed with dark brown hairs of moderate

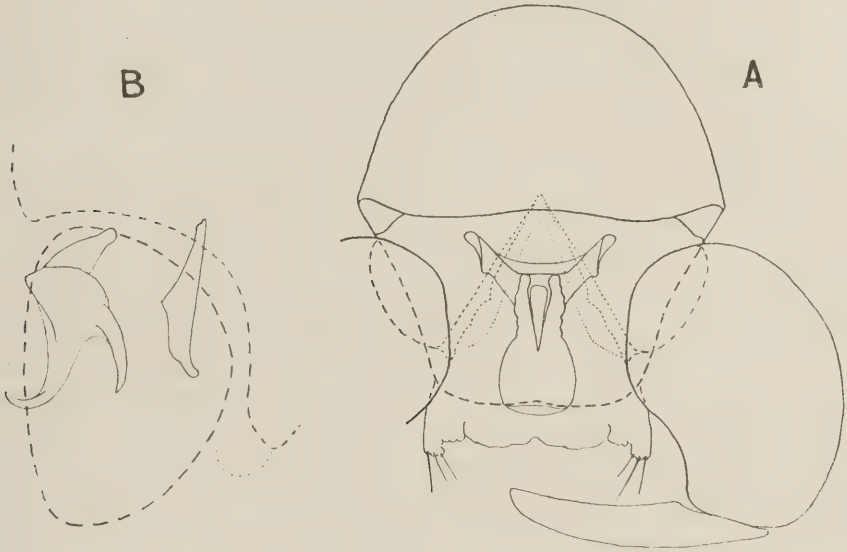


FIG. 5. *Forcipomyia auripes*, sp.n. ♂: Diagrams of hypopygium. *A*—Ventral view, *B*—Lateral view to show the relative positions of the aedoeagus and the dorsal process arising at the base of the side-piece. × *c.* 265.

length; sternite not notched in the middle line posteriorly. Forceps: side-pieces broad, uniformly very dark brown, clothed with numerous dark hairs some of which are of great length and others very stout and strong; claspers dark brown, shaped like the blade of a scythe, pubescent to the tips. Harpes: the rod-like structures which arise dorsally at the bases of the side-pieces converge anteriorly and meet in a sharp point; these rods are of dense chitin, but are extended internally into a narrow strip of delicate chitin. Aedoeagus

in ventral view showing a broad basal arch which is well-chitinised, and a posterior extension of delicate chitin with a rounded, slightly everted end.

GOLD COAST: Accra, 1920, 1 ♂, taken in the laboratory.

The position of this species is somewhat obscure. We have placed it in the Genus *Forcipomyia* because many of its characters are those of that genus, but it should be noted that the genitalia are not of the common type, and that the length of the costa and the form of the radial cells are unusual and more closely resemble those of the Genus *Lasiohelea*.

### *FORCIPOMYIA SQUAMIPENNIS*, sp.n.

Length of body (both sexes), about 2·0 mm.; length of wing in the female, 1·4 mm., in the male 1·3 mm.; greatest breadth of wing in the female 0·4 mm., in the male 0·3 mm.

*Head* dark brown; occiput clothed with long, dark brown hairs intermixed with which are numerous rather short, striated scales. Eyes bare, broadly contiguous above in both sexes, the facets separated by a narrow line. Clypeus, proboscis, and palpi dark brown. Clypeus bearing about sixteen hairs. Palpi similar in both sexes; second, fourth, and fifth segments sub-equal, the last two rather broadly united, third segment longer than the fourth and fifth together, inflated in its middle third, and furnished with a shallow and not unusually large sensory pit. *Antennae* (fig. 6, c) brown. In the female segments 3 to 10 bearing long, slender spines, not much thicker than the hairs, as long as the segments, and tapering to rather sharp ends; and whorls of about twelve hairs. All the flagellum segments bear also short, and moderately short, spines. First segment rather large, petiolate, bearing numerous stout, dark brown, pubescent hairs, the longest of which project beyond the distal border of the torus. Torus sub-spherical, darker brown than the flagellum, bearing about a dozen hairs. Third segment with a quite short stalk. Segments 4 to 10 flask-shaped, almost sub-equal, length from about twice to two-and-a-half times the greatest breadth. Segments 11 to 15 sub-cylindrical, 11 to 14 sub-equal, about three times as long as broad, with a distinct 'collar'; the last segment

a little longer, about four times as long as its basal breadth, ending in a blunt stylet. The combined length of segments 11 to 15 (128 units) rather less than that of segments 4 to 10 (145 units). In the male segments 3 to 11, generally, similar to those of *F. castanea*, the eleventh measuring about 16 units by 8 ; plume large, with its distal half tawny brown. Segments 12 to 15 elongated, their lengths measuring in one specimen 57, 34, 28 and 28 units respectively, the last segment ending in a blunt stylet. The combined length of segments 12 to 15 (147 units) almost equal to that of segments 3 to 11 (142 units), and greater than that of segments 4 to 11 (119 units). *Thorax* uniformly darkish brown ; well-clothed dorsally with light brown hairs and narrow scales, and with longer and darker hairs marginally and posteriorly. Pleura brown, paler than the dorsum. Scutellum dark brown, bearing an irregular submarginal row of about twelve to fifteen large, pale brown bristles, and numerous (twenty-five to fifty) small hairs and scales ; the number of bristles and hairs in the male is smaller than in the female. Post-scutellum dark brown, bare. *Wings* in both sexes covered with the usual minute microtrichia and densely clothed all over with small, rather broad scales. In the female dark grey, with a number of rather ill-defined, cream-coloured spots arranged in much the same manner as those on the wings of *Culicoides*. Two pale spots are situated on the anterior half of the wing, the one just beyond the junction of the third vein with the costa, and the other, a large, diffuse spot, upon the apical fourth ; there is also some indication of a pale spot covering the cross-vein, and the base of the wing is somewhat pale. Along the margin on the posterior half of the wing are four ill-defined pale spots, one each, between the rami of the fourth vein, between the fourth and fifth veins , between the rami of the fifth vein, and in the anal angle. The wing is darkest anteriorly, especially at the junctions of the first and third veins with the costa, and is clothed with scales, those upon the cream-coloured spots being of a yellow colour, those upon the darker portions of the wing being dark grey or dark brown. The scales are especially dense, broad, and dark upon the costa and the first and third veins. In the male the wings are narrower, less densely covered with scales, and a lighter colour, so that they appear to be pale, with darker markings, and not the reverse as in the female. Fringe long, composed of lanceolate

hairs which are delicately fringed. Costa reaching to about the middle of the wing. First and third veins fused basally, the proximal cell obsolete ; second radial cell (fig. 6, A) large, about three times as long as broad (external dimensions). Petiole of fourth vein longer than the cross-vein ; proximal ends of the rami almost obsolete. Bifurcation of the fifth vein in both sexes a little proximal to the level of the end of the costa. Halteres with dull white knobs. *Legs* in both sexes conspicuously banded, yellow and dark brown; femora, tibiae, and tarsi densely clothed with scales in addition to hairs. Coxae of all the legs dark brown. Femora with a broad dark brown band at base and apex, the bands being larger in the female than in the male, and the apical band in the male sometimes divided into two parts. Fore and middle tibiae with broad dark brown basal and apical bands each covering about one-third of the segment ; hind tibiae with the basal third dark brown and the apical two-thirds yellow with a narrow dark brown band in its middle. The knees, however, are usually yellow. First tarsal segment with the basal two-thirds dark brown and the apical third yellow ; second, third, and fourth segments more or less infuscated dark brown, in the middle ; fifth entirely yellow. Middle tibiae with a transverse row of apical spine-like hairs, some of which may be nearly as long as the first tarsal segment. In both sexes the first tarsal segment on the fore legs is longer than the second (about 4 : 3), on the middle legs slightly shorter (about 11 : 12), and on the hind legs distinctly shorter (about 5 : 7). Claws small, less than half the length of the fifth tarsal segment ; apparently not bifid in the male. Empodium about as long as the claws, hairy. *Abdomen* in the female uniformly dark brown, densely clothed with broad scales and with dark and light brown hairs, a tuft of hairs on the posterior extremity being especially long. Cerci pale brown. In the male pale, yellowish, longer and narrower than in the female, with dark brown bands near the posterior margins of the segments, which are broadest ventrally and towards the posterior end of the body ; the dorsal aspect of the proximal half of the abdomen almost entirely yellow, and the ninth segment entirely dark brown. Abdomen well-clothed with scales and hairs, the latter particularly long and numerous on the ventral surface. Spermathecae (fig. 6, D) two, very highly chitinised, sub-equal, pyriform, with a rather long conical portion

leading to the duct; greatest breadth about 45$\mu$ to 50$\mu$, and total length about 80$\mu$ to 85$\mu$.

Hypopygium (fig. 6, e and f). Ninth segment dark brown; tergite very short, sternite with numerous hairs and scales. Forceps: side-pieces with dark brown apices and paler, yellowish bases; claspers rather densely chitinised basally, their ends somewhat expanded, spatulate, but not hatchet-shaped. Harpes very highly chitinised, shaped like strong hooks, crossing in the middle line, with their extremities bent ventrally and tapering to a point.

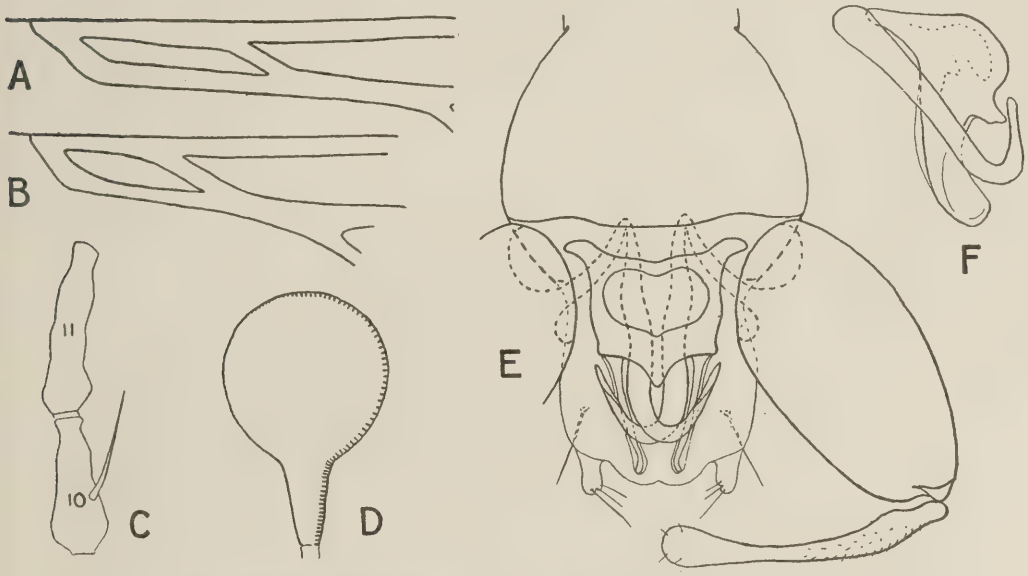


Fig. 6. *Forcipomyia squamipennis*, sp.n. *A*—Distal radial cell of wing of ♂, *C*—Tenth and eleventh segments of antenna of ♀, *D*—Spermatheca of female, *E*—Hypopygium of ♂, ventral view, *F*—Harpes and aedoeagus, lateral view. *Forcipomyia lepidota*, sp.n. *B*—Distal radial cell of wing of ♂. *D* × *c.* 400; others × *c.* 265.

Aedoeagus very highly chitinised in parts, having a ventral shield-like portion, the posterior edge of which is almost black and is produced into a median and two lateral projections, and on each side a little more dorsally, a strong, ventrally curved, rod-shaped structure.

Gold Coast: Accra, 1920, ♂♂ and ♀♀; Aburi, 1912-13, 2 ♀♀ (W. H. Patterson).

It is not improbable that *F. chrysolopha* (K.) is identical with this or one of the three following species, but this fact can only be determined by a re-examination of the type materials, particularly the genitalia of the males.

### *FORCIPOMYIA LEPIDOTA*, sp.n.

This species resembles the preceding one, *F. squamipennis*, in almost every respect excepting in the structure of the hypopygium of the male. The only points of distinction, and some of these may not be of any importance, since they are in characters which in other species are liable to variation, that we are able to detect after a careful comparison are as follows: The distal end of the antennal plume is pale, but not tawny; the terminal segments (11 to 15) of the antenna of the male measure in length (average of four specimens) about 14, 37, 22, 16, and 22 units respectively, the fourteenth segment, therefore, about one-fourth shorter than the fifteenth, and not about equal to it as in *F. squamipennis*. Scales on the wings smaller than in *F. squamipennis*, those on the surface of the wing narrower, more like lanceolate hairs. Fringe hairs lanceolate, but apparently simple. Distal radial cell (fig. 6, B) shorter. Bifurcation of the fifth vein slightly distal to the level of the end of the costa. Femora with dark bands broader than in *F. squamipennis*, occupying the greater part of the segment. Hind tibiae with basal dark-brown band somewhat narrower, and the second dark brown band somewhat broader than in *F. squamipennis*, so that the segment is divided into four areas of about equal length, alternately dark brown and yellow. The structure of the hypopygium is, however, quite unlike that of *F. squamipennis*.

HYPOPYGIUM (fig. 7, A and B). Ninth segment dark brown, not so densely covered with hairs as in *F. squamipennis*. Forceps: side-pieces dark brown, slightly paler brown or yellowish at the apex; claspers pale brown, rather delicately chitinised, with the distal extremity greatly expanded. Harpes apparently partly enfolded in the membranous portion of the aedoeagus, and difficult to see clearly in a fresh specimen. After treatment with caustic potash appearing as long, stout, strongly chitinised rods, tapering distally, and ending rather bluntly. Aedoeagus a complicated structure only partly, and not very highly chitinised; in ventral view the most characteristic features are a basal, stirrup-like portion with a median ventral process, and a distal, shuttle-like structure.

GOLD COAST: Accra, 1920, numerous specimens; Sekondi, July 1922, 3 ♂♂, 1 ♀, in a bungalow (Dr. J. F. Corson).

Owing to the similarity of this species to several others obtained at Accra which, apart from the structure of the hypopygium of the male, are almost, if not quite, indistinguishable, it has not been possible to decide with certainty which, if any, of the females of this type collected by us should be associated with it. A large number of females were collected with the males, all of which are practically identical, apart from variations in size which do not appear to be of importance. We are unable to separate them into different

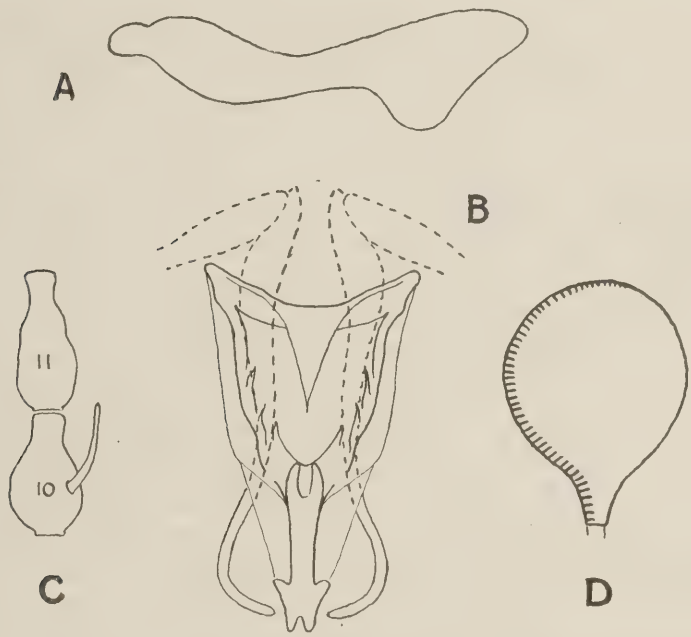


FIG. 7. *Forcipomyia lepidota*, sp.n. *A*—Outline of clasper of ♂, *B*—Diagram of harpes and aedoeagus of ♂, ventral view, *C*—Tenth and eleventh segments of antenna of ♀, *D*—Spermatheca of ♀. *D.* × *c.* 600; others × *c.* 400.

species as has been done in the case of the males, and it must therefore be left for future investigation to decide whether they represent one or several species. In the meantime a description of the female should be given, and we propose to give it here, because the male *F. lepidota* was abundant in our material and it therefore appears to us probable that the female also was abundant, and because one such female was taken in a bungalow at Sekondi together with three males

of *F. lepidota*, the only species of this group hitherto collected in that locality. It must be clearly stated, however, that with equal or almost equal justice it might be claimed that the description is also that of the females of the two following species (*F. venusta* and *F. pampoikila*), and that we are unable to detect any differential characters of specific importance between the female reared from banana fibre together with a male of *F. venusta*, and therefore presumably the female *F. venusta*, and the female taken at Sekondi, together with three males of *F. lepidota*, and therefore presumably the female *F. lepidota*.

FEMALE. Length of body, 1·3 mm. to 1·8 mm.; length of wing, 0·8 mm. to 1·0 mm.; greatest breadth of wing, 0·3 mm. to 0·4 mm.

*Head* similar to that of *F. squamipennis*, but hairs on clypeus apparently fewer, five to seven, and third segment of the palp furnished with a deeper sensory pit. *Antennae* (fig. 7, C) brown. Large spines on segments 3 to 10 more or less geniculate, twice as stout as the hairs, shorter than the segments, with blunt ends. Flagellum segments forming an almost continuous series, as in *F. squamipennis*, but more distinctly. Segments 4 to 10 slightly constricted apically, but not so clearly flask-shaped as in *F. squamipennis*, sub-equal, less than twice as long as broad, the length and greatest breadth of segments 4 and 10 being in one example 9 by 7, and 10 by 6 units, respectively. Segments 11 to 15 elongated, sub-cylindrical, 11 to 14 sub-equal, about twice as long as broad (13 by 5 units), the last segment a little longer (17 units), about three times as long as broad. The combined length of segments 11 to 15 slightly less than that of segments 4 to 10, in one specimen 74 to 77 units. *Thorax* similar to that of *F. squamipennis*. *Wings* similar to those of *F. squamipennis*, but scales on the general surface narrower, as in the male. Distal radial cell as in the male *F. lepidota*. Bifurcation of the fifth vein slightly proximal to the level of the end of the costa. *Legs* similar to those of *F. squamipennis*; the hind tibiae adorned as in the male *F. lepidota*. *Abdomen* similar to that of *F. squamipennis*, but the scales rather narrower. Spermathecae (fig. 7, D) two, similar to those of *F. squamipennis* but smaller (average length about 60$\mu$, and greatest breadth about 40$\mu$), and with the proximal conical portion less largely developed.

### *FORCIPOMYIA VENUSTA*, sp.n.

This species resembles *F. lepidota* so closely, that apart from the characters of the hypopygium of the male we are unable to separate the two with certainty. The measurements of the terminal segments (11 to 15) of the antenna of the male averaged in four specimens 15, 44, 26, 20, and 22 units in length respectively. The banding of the hind tibiae is as in the preceding species, *F. lepidota*. The structure of the hypopygium is, however, quite distinctive.

Hypopygium (fig. 8). Ninth segment dark brown; sternite rather more hairy than in *F. lepidota*. Forceps: side-pieces and claspers similar to those of *F. lepidota*. Harpes long, slender plates of chitin, not very highly chitinised, almost straight, tapering

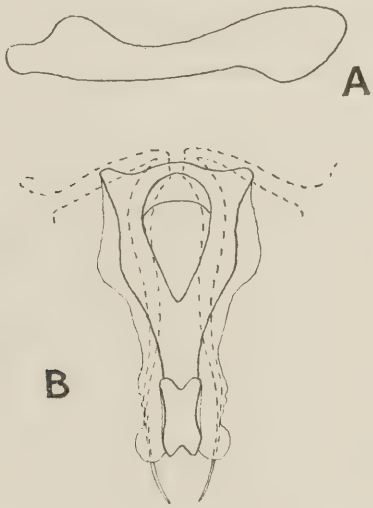


Fig. 8. *Forcipomyia venusta*, sp.n. ♂: *A*—Outline of clasper, *B*—Diagram of harpes and aedoeagus, ventral view.

distally and ending in sharp points. Aedoeagus similar to that of *F. lepidota*, but stirrup-shaped portion more uniformly chitinised, as shown in the figure.

Gold Coast: Accra, 1920, numerous specimens taken on the laboratory windows; Nsawam, near Accra, 26.5.1920, 1 ♂, 1 ♀, reared from rotting banana fibre.

As in the preceding species, we have not been able to separate with certainty the female of this insect from those of allied species (see *F. lepidota*).

### *FORCIPOMYIA PAMPOIKILA*, sp.n.

This species, like the preceding one, resembles *F. lepidota* so closely that apart from the characters of the hypopygium of the male we are unable to distinguish them with certainty. The measurements of the last five segments (11 to 15) of the antenna of the single male in our possession are approximately 14, 38, 23, 21, and 24 units respectively. The banding of the hind tibiae is similar to that in *F. lepidota*. The hypopygium is characteristic.

Hypopygium (fig. 9). Ninth segment dark brown, closely covered with hairs both dorsally and ventrally. Forceps: side-pieces dark brown at base and apex, with a paler, yellowish band

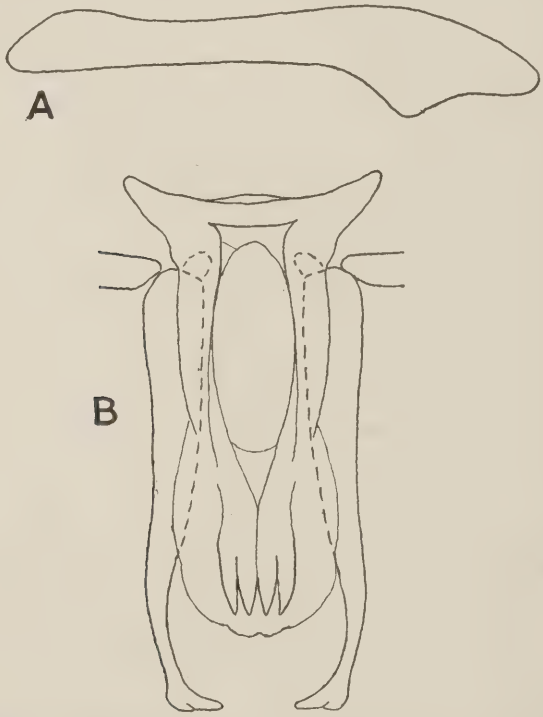


Fig. 9. *Forcipomyia pampoikila*, sp.n. ♂: *A*—Outline of clasper, *B*—Diagram of harpes and aedoeagus, ventral view. × *c.* 400.

about the middle; claspers pale brown, not very highly chitinised, only slightly expanded at the end. Harpes long, slender plates of chitin, almost straight, tapering distally and with ends slightly

expanded and shaped like the head of a duck. Aedoeagus of the usual form but mainly membranous or but feebly chitinised, distal end showing in ventral view four short chitinised processes.

GOLD COAST: Accra, 1920, 1 ♂, taken in the evening upon a window of the laboratory.

### *FORCIPOMYIA NIGROTIBIALIS*, sp.n.

Length of body (one female), 1·6 mm.; length of wing, 1·2 mm.; greatest breadth of wing, 0·4 mm. A very dark brown, almost black, species, with dark brown tibiae on the middle and hind legs; with scales. We have in our possession only a single specimen of this insect, and this unfortunately was partially destroyed by Psocids before our examination was completed, and in consequence our description is in some particulars deficient.

*Head* very dark brown. Proboscis and palpi dark brown. *Antennae* dark brown, with short dark brown hairs. Basal segments missing. Segments 7 to 10 oval, slightly narrowed anteriorly, sub-equal, about 12 by 7 units; large spines pale brown, stouter than the hairs, a little longer than the segments, almost straight, tapering to pointed extremities; the whorls of about twelve hairs. Segments 11 to 15 more elongated, sub-cylindrical, 11 to 14 sub-equal, about 15 by 6 or 7 units, the last segment a little longer, about 21 units, ending in a short, blunt stylet. *Thorax:* Dorsum very dark brown. Pleura very dark brown above, paler brown below. Scutellum very dark brown; bearing a transverse row of seven large bristles, and about ten smaller bristles and hairs. Post-scutellum very dark brown. *Wings* unadorned, but the anterior margin somewhat darker than the rest of the wing, and the extreme base yellowish; well-clothed with dark hairs and narrow scales. Microtrichia minute. Fringe as usual; long hairs slightly curved, slender, apparently simple. Costa reaching to, or very slightly beyond, the middle of the wing. First radial cell obsolete or nearly, second well-formed. Bifurcation of the fifth vein at about the same level as the end of the costa. Halteres with yellowish knobs. *Legs* conspicuously marked, well-clothed with hairs and narrow scales, and bearing (especially on the femora and tibiae) large hastate spines with long tapering ends. Fore legs nearly

uniformly light brown. Middle legs with femora and tibiae very dark brown, excepting at the base of the femora and the apex of the tibiae, where they are light brown; tarsal segments light brown. Hind legs with apical half of femora light brown, basal half very dark brown; tibiae very dark brown, excepting at the extreme apex, which is slightly tawny; tarsal segments light brown. First tarsal segment of the middle and hind legs shorter than the second (about 35 : 41 and 40 : 49 units), that of the fore legs about equal to the second (about 36 : 33 units). Claws simple, equal, less than half the length of the fifth tarsal segment. Empodium well-developed, hairy. *Abdomen* very dark brown; densely clothed with dark brown scales. Spermathecae two, highly chitinised, sub-spherical, sub-equal, diameter about 50$\mu$ to 60$\mu$; the commencement of the duct chitinised for a very short distance, about 4$\mu$.

GOLD COAST: Accra, 1 ♀, taken in the evening upon a window in the laboratory.

### *FORCIPOMYIA INORNATIPENNIS* (Austen).

Length of body, 2·1 mm. to 3·1 mm.; length of wing, 1·4 mm. to 1·9 mm.; greatest breadth of wing, 0·4 mm. to 0·7 mm. The size varies greatly in both sexes, and the males are rather longer than the females on account of the size of the hypopygium, and have longer and narrower wings. In different individuals the colour also varies considerably, from brown to very dark brown, and females are usually darker than males. Long, slender, striated scales present.

*Head* brown to very dark brown; occiput clothed with yellowish or dark brown hairs. Eyes bare; broadly contiguous above in both sexes. Clypeus, proboscis, and palpi dark to very dark brown. Clypeus bearing about six to eight dark brown hairs. Mouth parts similar to those of *F. castanea*, but the mandibles of the female more slender and pointed at the ends, and armed with more numerous teeth (about fifty to sixty), those in the middle of the series being rather large; teeth on the maxillae also more numerous, about twenty-five. Palpi (fig. 10, A and B) in both sexes with second and fourth segments sub-equal, fifth small, not much more than half the length of the fourth, and third much the longest, twice as long as the fourth. In the female the third segment in its middle three-fourths is greatly inflated on its inner aspect, and is furnished with a very

large, oval, sensory pit with a relatively small anterior opening near which, on the surface of the segment, are a number of sensory hairs; in the male the third segment is slender, inflated to a lesser extent in its middle third, and furnished with an oval sensory pit of moderate size. *Antennae* brown to darkish brown. In the female segments 3 to 10 bearing moderately stout, slightly curved spines, as long as the segments, colourless, with pointed ends; and whorls

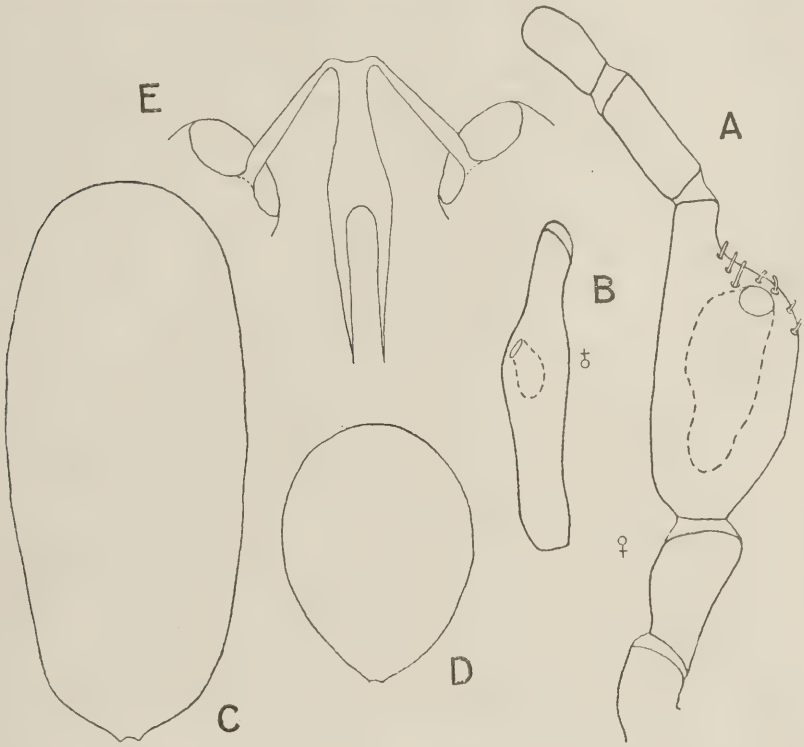


Fig. 10. *Forcipomyia inornatipennis* (Aust.). *A*—Palp of ♀. *B*—Third segment of palp of ♂. *C* and *D*—Spermatheca of ♀ to show variation in size and shape. *E*—Harpes of ♂, ventral view. *A* and *B* × *c.* 265; *C* and *D* × *c.* 400.

of about twelve hairs. The first segment rather large. The basal segments of the flagellum sub-equal, from sub-spherical to oval, the fourth and the tenth in one specimen measuring 12 by 12, and 15 by 9 units respectively. Segments 11 to 15 sub-cylindrical, elongated, 11 to 14 a little over four times as long as broad (about 30 by 7 units), with a distinct 'collar,' the fifteenth rather longer (about 43 by 7 units) and ending in a blunt stylet. The combined length of

segments 11 to 15 greater than that of segments 3 to 10, or 4 to 10, *e.g.* in one specimen measuring 165 units as compared with 113, or 98 units. In the male similar to those of *F. castanea*, but the terminal segments longer, the lengths of the last five segments in one specimen measuring 18, 65, 43, 38, and 44 units respectively. The combined length of segments 12 to 15 greater than that of segments 3 to 11, *e.g.* in one specimen 190 units as compared with 152 units. *Thorax* almost uniformly brown to very dark brown, shoulders rather paler brown; clothed with longish yellow hairs and short brown to yellowish hairs, and bearing also numerous small scales. Pleura a rather lighter brown than the dorsum. Scutellum and post-scutellum dark brown to almost black, the former bearing numerous bristles and hairs which in the female are more abundant than in the male. *Wings* rather densely clothed with dark brown lanceolate hairs, and bearing numerous long, striated scales on the costa and the first and third veins, which are very dark and dense, and give the wing in this region a darker appearance than elsewhere. There is no pale spot in the middle of the wing as in *F. castanea*, but the extreme base is somewhat lighter coloured than the rest. The wing surface is slightly infuscated in the neighbourhood of the radial cells and the adjacent intercalary vein and along the rami of the fourth and fifth veins. First and third veins fused basally, the first radial cell therefore obsolete, but the second well-formed. Costa extending to about the middle of the wing. Bifurcation of the fifth vein a little proximal to the level of the end of the costa in both sexes. In the male the wing is paler, less densely clothed with hairs and scales, longer, and narrower than in the female. Halteres with cream-coloured knobs. *Legs* yellowish-brown, the terminal tarsal segments somewhat infuscated; rather hairy and bearing also numerous scales. The hind legs may be entirely without dark bands, or may have well-defined dark brown bands on each side of the knees as in *F. biannulata*, or on the femora only; the middle legs may also have similar bands, but they are usually less well-developed than on the hind legs. The presence or absence of the bands on the legs appears to depend on the depth of general colouration of the specimen, the darker individuals showing the greater development of the banding on the legs. In the male, the first tarsal segment is about half the length of the second on the fore and hind legs; shorter, about

one-third the length of the second, on the middle legs ; in the female, the first tarsal segment is about two-thirds the length of the second on the fore legs, three-eighths on the middle legs, and a half on the hind legs. Claws and empodium similar to those of *F. castanea*. *Abdomen* uniformly brown to dark brown in the female, densely clothed with brown and golden-brown hairs, and long, striated scales which are particularly dense on the lateral aspects ; in the male paler, the proximal segments pale brown with more or less complete, broad, dark brown bands, and sparsely clothed with long, dark brown and golden-brown hairs, and bearing fewer scales. Hypopygium very dark brown. Cerci of the female paler than the rest of the abdomen. Spermathecae (fig. 10, C and D) two, very highly chitinised, oval, often unequal and rather variable in size, those measured ranging in length from 80$\mu$ to 130$\mu$, and in breadth from 60$\mu$ to 80$\mu$ ; practically no part of the duct is chitinised.

HYPOPYGIUM similar to that of *F. castanea* but more highly chitinised and darker brown. Ninth segment similar to that of *F. castanea*. Forceps similar to those of *F. castanea* but darker ; claspers dark brown. Harpes (fig. 10, E) united by their basal halves, fork-like, the prongs pointed but not filiform. Aedoeagus similar to that of *F. biannulata*.

LARVA (fig. 11). A gravid female taken in the Accra laboratory on the 26th June, 1921, and imprisoned in a tube containing vegetable débris was observed on the following day to have laid eggs. Fifteen days later numerous larvae were found in the tube, but unfortunately none of them pupated, and six days later (18th July) it was found that all were dead. The dead larvae were preserved and are briefly described here. They are small, length about 2 mm., and presumably not fully grown ; dull white in colour, and armed with brownish setae and hairs. *Head* brown, without definite colour pattern. Macrochaetae *p* and *q* long, slender, slightly barbed, tapering to sharp points ; *p* longer than *q*. Antenna terminating in a small spine which, however, is longer than the corresponding spine in *F. castanea*. *Body :* admedian dorsal spines (*a*) small, hastate on segments 3 to 8, but much reduced on the other segments. On the fifth and sixth segments they are largest ; length about 78$\mu$ ; length and breadth of the terminal, spear-like portion about 33$\mu$ and 22$\mu$ respectively, the point not drawn out as in *F. castanea*. Dorso-

lateral setae (*b* and *d*) arising from a large double tubercle, both stout, brown, barbed setae; *d* straight and pointed, *b* longer, curved, and blunter. Macrochaeta *c* similar to *d* but shorter and slightly curved, arising from a conical tubercle. Ventro-lateral hairs (*e* and *f*) long and slender, arising from separate tubercles. Prothoracic pseudopod similar to that of *F. castanea*, but the crowns

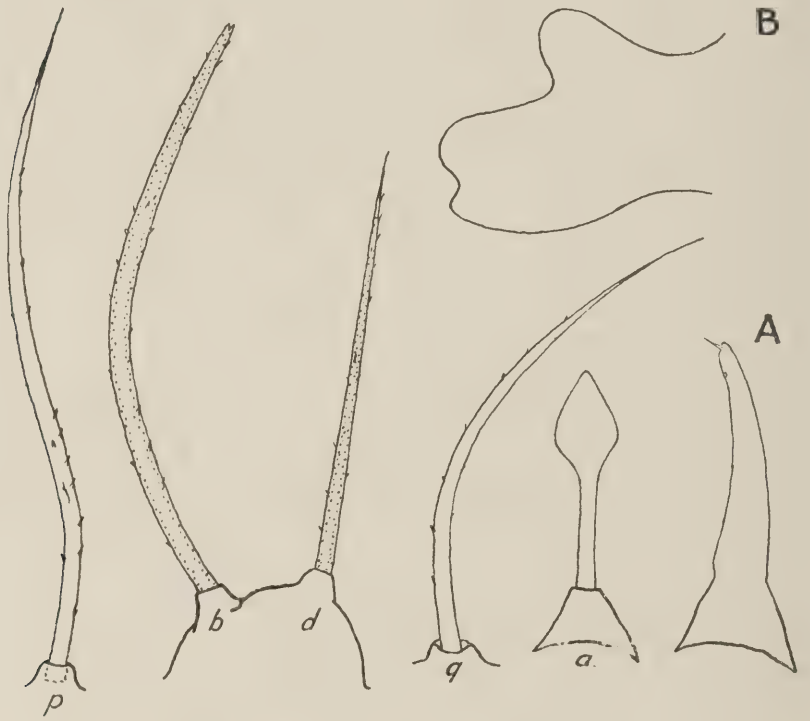


FIG. 11. *Forcipomyia inornatipennis* (Aust.). Larva: *A*—Antenna, *B*—Anal blood-gill of one side, ventral view; *p*, *q*, *a*, *b*, and *d*—macrochaetae lettered according to Saunders. *B* × *c.* 600; rest × *c.* 400.

composed of only five hooks each. Anal blood-gills bilobed. Posterior pseudopod armed with eighteen hooks in two rows, nine on each side, as in *F. castanea*.

GOLD COAST: Accra, numerous specimens, both males and females, taken in the evening on the windows of the laboratory and in bungalows at all times of the year. Obuasi, Ashanti, April, 1920, 1 ♂ (Dr. A. S. Burgess).

NIGERIA: Lagos, June, 1921, 3 ♂♂, 6 ♀♀ (Dr. H. A. Foy); Oshogbo, November 1910, 1 ♂ (Dr. T. F. G. Mayer); Yaba, near Lagos, 20.5.1909, 1 ♂ (Dr. W. M. Graham), 'caught on hand near lamp at 9 p.m.'

TANGANYIKA: Dar-es-Salaam, 16.4.1920, 1 ♀ (W. C. C. Pakes), 's.s. Prinzessin, one mile from shore.'

This species was originally described by Austen (1912) from a single female collected near Lagos. The large number of specimens in our possession have enabled us to describe the male and to add some points of importance with regard to the female. Perhaps our most important observation is that the species is variable and that individuals which are otherwise identical and which have indistinguishable genitalia may have either entirely unbanded legs, banded hind legs only, or banded middle and hind legs. Such characters as the colour markings of the legs without confirmation by morphological differences are, therefore, insufficient for specific distinction (see also *F. castanea*). The forms of *F. inornatipennis* with banded legs can only be regarded as a variety, and as it will be convenient, we propose to refer to them as *F. inornatipennis*, var. *ornaticrus*.

### *FORCIPOMYIA HIRSUTA*, sp.n.

Length of body (one male and two females), 1·6 mm. to 2·2 mm.; length of wing, 1·3 mm. to 1·5 mm.; greatest breadth of wing, 0·4 mm. The body and the wings in the male are slightly longer than in the female. A very hairy, darkish brown species with banded legs.

*Head* darkish brown, occiput clothed with long, dark brown hairs. Eyes bare; in the female broadly, in the male more narrowly contiguous above. Clypeus, proboscis, and palpi brown; clypeus and palpi bearing rather numerous and long dark brown hairs. Palpi (fig. 12, A) with a rather large first segment; second, fourth and fifth segments sub-equal, about as broad as long, the fourth nearly conical and broadly connected with the fifth; the third segment longer than the fourth and fifth together, inflated basally, with a somewhat variable sensory pit which is sometimes divided into one or two small distal sensory pits in addition to the usual larger proximal one—in the male the sensory pit is reduced. *Antennae:*

In the female first segment rather large, darkish brown, bearing rather numerous, long, stout, dark brown hairs. Torus darkish brown. Segments 3 to 10 quite colourless basally, and dark brown apically, the change of colour taking place abruptly at the level of the whorl of hairs ; segments 11 to 15 entirely dark brown. Segments 3 to 10 bearing long spines, which are almost as long as the segments, twice as stout as the hairs, and with pointed extremities ; and whorls of about fourteen dark brown hairs. Segments 4 to 10 more or less flask-shaped, sub-equal, but gradually becoming a little longer, narrower, and more attenuated distally ; the actual measurements of length and breadth of the fourth and tenth segments in one specimen being 15 by 9 and 19 by 8 units respectively. Segments 11 to 14 sub-cylindrical, tapering a little distally, sub-equal, a little more than three times as long as their basal breadth ; segment 15 rather larger, about five times as long as broad, not tapering distally but ending in a rather long stylet. The combined length of segments 11 to 15 slightly greater than that of segments 4 to 10, but less than that of segments 3 to 10, the actual measurements in one specimen being 129, 124, and 141 units respectively. In the male the first segment a mere ring of chitin ; the torus very large, yellowish-brown ; the flagellum segments pale brown, but the last three darker than the others ; and the plume dark brown basally and pale brown apically. General form of the flagellum segments as usual ; the lengths of segments 11 to 15 in one specimen being 18, 67, 40, 30, and 33 units respectively. The combined length of segments 12 to 15 almost equal to (slightly greater than) that of segments 3 to 11. *Thorax :* Dorsum uniformly darkish brown, glossy, rather sparsely clothed with dark brown hairs, some of which are arranged so as to form a curved row on each side immediately in front of the scutellum ; pleura paler, yellowish brown. Scutellum dark brown at the sides, a little paler brown in the middle ; bearing in both sexes about twenty to twenty-five bristles and hairs, of which ten are especially large. Post-scutellum dark brown, with a small depression posteriorly. *Wings* brownish, darker in the female, without spots, covered with microtrichia as usual, densely clothed with decumbent hairs, and bearing on the costa and the first and third veins numerous dark brown, lanceolate scales ; the hairs are most abundant near the anterior border of the wing and with the dark scales give the appear-

ance of a dark line in this region when examined with a hand lens. Fringe long. Costa reaching to the middle of the wing in both sexes. First and third veins fused basally, but forming distally a cell of some size. Bifurcation of the fifth vein at about the level of the end of the costa in the male, slightly proximal to it in the female. Halteres with buff-coloured knobs. *Legs* yellowish-brown, with darker brown bands at the apex of the hind femora, the base of all the tibiae, and the apex of the fore tibiae and about the middle of the hind tibiae; tarsal segments on all the legs infuscated. The darker markings are less distinct in the male than they are in the female. Legs very

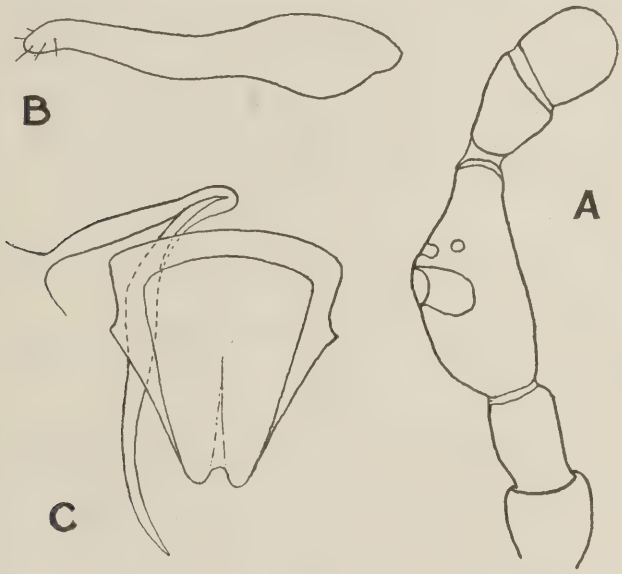


FIG. 12. *Forcipomyia hirsuta*, sp.n. *A*—Palp of ♀. *B*—Clasper of ♂. *C*—Diagram of aedoeagus and one harpe of ♂, ventral view. × *c.* 400.

hairy, the tibiae in particular bearing very long hairs dorsally, some of them almost as long as the segment; bearing also broad scales on the femora and the distal segments. First tarsal segment in both sexes longer than the second on the fore and middle legs, very slightly shorter than the second on the hind legs. Claws and empodium as usual, but the claws in the male do not appear to be bifid. *Abdomen* darkish brown in the female, paler brown in the male and with darker brown markings especially on the posterior segments; in both sexes very hairy posteriorly, and bearing also

numerous scales. Hypopygium of the male brown. Spermathecae two, highly chitinised, sub-equal, pyriform, about 65$\mu$ by 45$\mu$, the proximal end tapering to merge with the duct.

HYPOPYGIUM (fig. 12, B and C). Ninth segment of the usual form, hairy, lateral parts of the posterior extension of tergite slightly chitinised; Forceps: side-pieces oval, short and broad, densely clothed with dark brown and yellowish hairs, some of which are extremely long; claspers darkish brown, not expanded at the end. Harpes blade-like, long, narrow plates of delicate chitin, only slightly curved, tapering distally, with pointed extremities, somewhat resembling those of *F. venusta*. Aedoeagus feebly chitinised, but slightly denser at the sides and base than elsewhere, in ventral view triangular, with the apex notched.

GOLD COAST: Accra, 15.7.1920, 1 ♀., and 22.1.1921, 2 ♀♀, taken in the evening on the windows of the laboratory; Nsawam, near Accra, 19.11.1921, 1 ♂ and 1 ♀, reared from decaying banana fibre.

### *FORCIPOMYIA TIGRIPES*, sp.n.

Length of body (one male), 1·8 mm.; length of wing, 1·3 mm.; greatest breadth of wing, 0·3 mm. A light brown species with banded legs.

*Head* darkish brown. Eyes bare, broadly contiguous above. Clypeus, proboscis and palpi brown. Palpi similar to those of *F. castanea*. *Antennae* pale brown. Proximal segments similar to those of *F. castanea*, but the five distal segments (11 to 15) dissimilar, measuring respectively about 19 by 8, 35 by 7, 29 by 6, 23 by 6, and 31 by 7 units, the last segment without a basal expansion, rather broader than the fourteenth, and ending in a blunt stylet. The combined length of segments 12 to 15 less than that of segments 3 to 11, and a little less than that of segments 4 to 11. *Thorax* rather dark brown dorsally. Pleura pale brown. Scutellum and post-scutellum dark brown, the former bearing about sixteen bristles and a dozen small hairs. *Wings* long and slender, densely clothed with lanceolate hairs and bearing dark brown scales on the costa and the first and third veins. Wings a pale colour, with a small dark brown spot covering the second radial cell. Costa reaching not quite to the middle of the wing, first radial cell obsolete, second

well-formed; bifurcation of the fifth vein a considerable distance distal to the level of the end of the costa. Halteres with creamy-white knobs. *Legs* light brown with darker brown bands which are most distinct on the hind legs. Clothed with pale brown scales in addition to hairs. Bands on the hind legs covering the apical halves of the femora, the basal sixth and the middle third of the tibiae, the basal two-thirds of the first tarsal segments, and about the

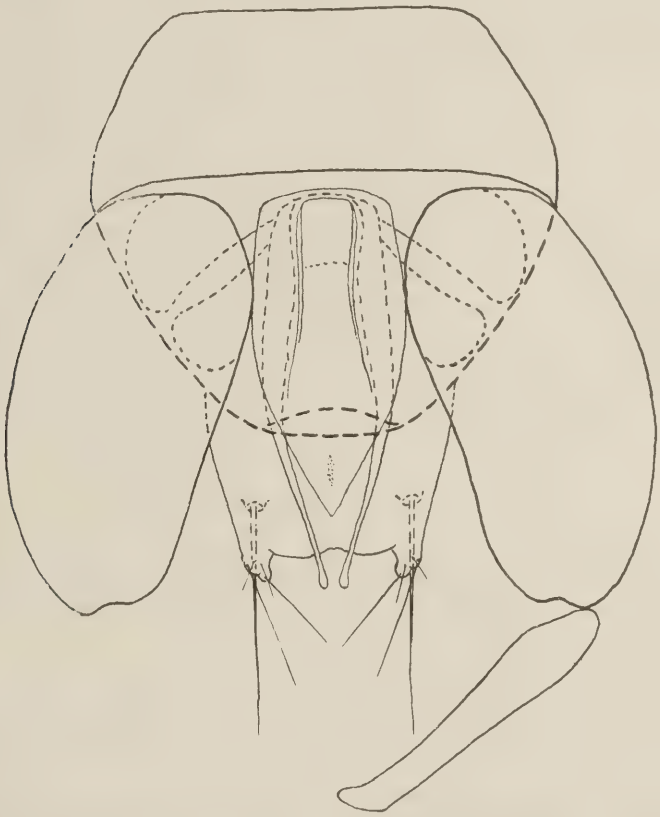

FIG. 13. *Forcipomyia tigripes*, sp.n. ♂: Diagram of hypopygium. × *c.* 400.

middle two-thirds of the second, third and fourth tarsal segments. The bands on the fore and middle legs much less distinct and smaller, the only conspicuous ones being on each side of the knees, and on the basal two-thirds of the first two tarsal segments. First tarsal segment about equal to the second (44 to 49) on the middle legs, but on the fore legs distinctly longer (45 to 35), and on the hind legs

distinctly shorter (45 to 54) than it. Claws small, not half the length of the fifth tarsal segment, not very strongly curved. Empodium long and hairy. *Abdomen* pale brown, with darker brown bands, often incomplete dorsally, on the middle parts of the segments; clothed with brown scales as well as hairs.

HYPOPYGIUM (fig. 13) dark brown. Ninth segment similar to that of *F. castanea:* sternite moderately hairy; membranous posterior extension of tergite bearing dorsally a pair of large sub-lateral bristles, and furnished with lateral terminal processes each bearing two long and two short hairs. Forceps: side-pieces short and broad (40 by 20 units), dark brown, bearing some very long stout hairs; claspers similar to those of *F. castanea*, not very dark, but infuscated at base and apex. Harpes long and slender, tapering distally but with their ends slightly expanded; they appear to be united across the middle line at the base by a narrow bridge of chitin. Aedoeagus rather long, with a conical posterior termination; mainly membranous, but chitinised laterally, and in ventral view showing on each side a sub-median longitudinal ridge of brown chitin.

GOLD COAST: Accra, 1920, 1 ♂, taken in the evening on a window in the laboratory.

### *FORCIPOMYIA AETHIOPIAE*, sp. n.

Length of body (one male), 1·1 mm.; length of wing, 0·9 mm.; greatest breadth of wing, 0·2 mm. A dark brown midge with hairy eyes; without scales.

*Head* dark brown. Eyes hairy; the facets rather widely separated, by about 18$\mu$. Clypeus, proboscis, and palpi brown. Clypeus bearing about six hairs. Palpi with first segment small, second and fourth sub-equal, not quite twice as long as broad, fifth small, shorter than the fourth, third about as long as the fourth and fifth together, slightly inflated in the middle, and furnished with a small sensory pit. *Antennae* darkish brown, the torus darker than the flagellum. Third segment smaller than the fourth, with a long (about 30$\mu$) petiole. Segments 4 to 11 of about equal length (10 to 9 units), but rapidly decreasing in breadth from 9 units in the case of the fourth, to 6 units in the case of the eleventh. Segments 12 to 15 elongated, measuring in length and greatest breadth about

27 by 6, 16 by 5, 13 by 5, and 17 by 7 units respectively ; segment 12 shaped like the eleventh, but with the distal end greatly drawn out, segments 13 and 14 sub-cylindrical with a small basal dilatation bearing a whorl of hairs, and segment 15 broader than the rest, tapering slightly distally, and ending in a blunt stylet. The combined length of segments 12 to 15 practically equal to that of segments 4 to 11. *Thorax* uniformly darkish brown dorsally, pleura paler brown. Scutellum dark brown, bearing two lateral and five centro-marginal bristles (two of the latter being small), and about seven

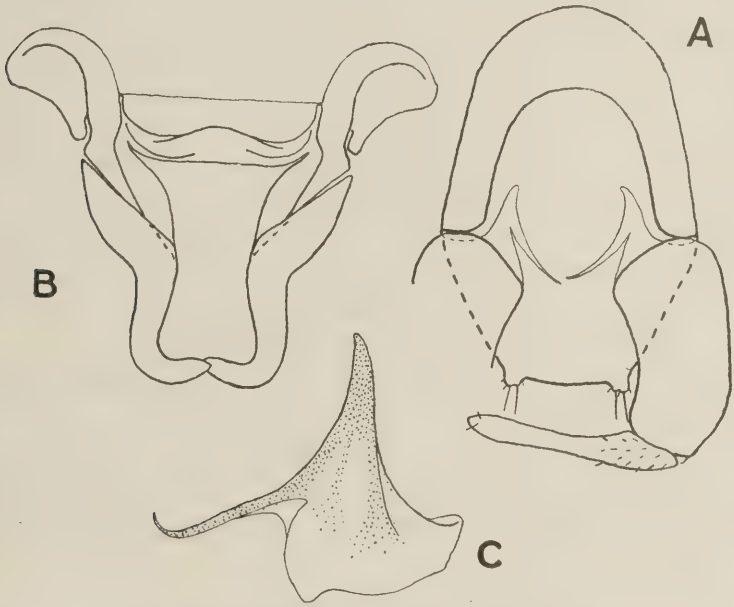


FIG. 14. *Forcipomyia aethiopiae*, sp.n. ♂ : Diagrams of parts of the hypopygium in ventral view : *A*—Ninth segment and forceps, *B*—Aedoeagus, *C*—Harpe. *A* × *c.* 375 ; *B* and *C* × *c.* 900.

smaller hairs Post-scutellum dark brown, darker than the scutellum. *Wings* unadorned, well-clothed with macrotrichia. Microtrichia minute. Frings hairs apparently simple. Costa reaching just beyond the middle of the wing (24 : 46). First radial cell obliterated, second well-formed, but not very long. Halteres with dull white knobs. *Legs* uniformly darkish brown. First tarsal segment more than twice the length of the second on all the legs, about three times the length on the fore and hind legs, rather shorter (36 : 16) on the

middle legs. Claws delicate, slender, more than half the length of the last tarsal segment, with bifid ends. Empodium long and hairy. *Abdomen* darkish brown, sparsely clothed with moderately long hairs.

Hypopygium (fig. 14). Ninth segment long, dark brown ; tergite densely clothed with long, stout hairs, its posterior margin rounded and bearing at each side a small irregularly shaped process armed with a few short hairs ; sternite very deeply excavated in the middle line posteriorly, the basal strip hairy. Forceps well-developed, side-pieces oval, slightly convex outwards, about twice as long as broad, and well-clothed with long, strong hairs ; claspers highly chitinised, dark brown, the basal third covered by small hairs. Harpes apparently represented by an irregularly triangular plate of chitin on each side with a thin process directed towards the middle line where it is contiguous with, but so far as could be made out not connected with, its fellow of the opposite side. Aedoeagus a very complex structure showing in ventral view a highly chitinised basal bar, and on each side projecting posteriorly a highly chitinised hook-shaped process, the two hooks directed inwards at their ends and sometimes overlapping, but not fused together.

Gold Coast : Accra, 1920, 1 ♂, taken on a window in the laboratory.

The systematic position of this species, of which we possess only a single male, is somewhat obscure, and it is placed here, with other species belonging to the genus *Forcipomyia*, provisionally only.

### *FORCIPOMYIA INGRAMI*, Cart.

Carter (1919) has described this species fully and has given excellent drawings of the more important parts, including a ventral view of the hypopygium of the male. The description of the latter, however, requires modification. At the same time, as we have at our disposal abundant material (including Carter's co-types), it may be of advantage to give additional or more exact details regarding certain parts which are now necessary for identification of the species.

The species is somewhat variable both in size and colour : usually dark brown as described by Carter, but sometimes much paler. Without scales. Eyes bare, apparently broadly contiguous, but

actually narrowly separated above in both sexes. The twelfth segment of the antenna of the male nearly three times as long as the eleventh and twice as long as the fourteenth, the thirteenth and fifteenth (including the stylet) sub-equal, a little longer than the fourteenth; the actual measurements of the last five segments (11 to 15) in three specimens of average size being approximately 14, 40, 29, 20, and 26 units respectively. The combined length of segments 12 to 15 slightly greater than that of segments 4 to 11, but less than that of segments 3 to 11. In the female occur whorls of about ten hairs and spines on the basal segments twice as stout as the hairs; the combined length of segments 11 to 15 slightly greater than that of segments 3 to 10, namely, about 84 to 75 units.

Scutellum in the male bearing about eight especially large bristles in a transverse row as in the female, but only about fifteen to twenty smaller bristles and hairs, that is about half the number in the female. In the male the costa reaching to the middle of the wing; bifurcation of the fifth vein distal to the level of the end of the costa. Halteres usually with white knobs in both sexes, sometimes with brown knobs, the pigment in them being in both cases white and the chitinous covering more or less infuscated. Fringe of the wing of the usual form, not, as shown in Carter's figures, a single row of hairs. First tarsal segment in the male slightly longer than the second on the fore legs, slightly shorter than the second on the middle and hind legs. Carter's statement that on the middle legs the first tarsal segment is slightly longer than the second is clearly an error, his co-type specimen showing the reverse relationship. Spermathecae (fig. 15, D) of the female, two, sub-equal, usually pyriform, with a terminal sub-spherical portion and a proximal conical portion leading to the duct, rather variable in size, but the greatest breadth usually about $50\mu$ to $60\mu$ and the total length about $65\mu$ to $85\mu$.

Hypopygium (fig. 15, A, B and C). Ninth segment darkish brown, large, the proximal end long, very hairy both dorsally and ventrally. Posterior border of the sternite not excavated in the middle. Tergite with a membranous posterior extension which reaches back as far as the ends of the side-pieces, and is strengthened by a narrow rod of chitin on each side, ending in a process bearing three or four hairs—this is the process referred to by Carter as the basal lobe of the side-pieces. Forceps: side-pieces dark brown, not quite twice as

long as broad, almost reniform, with a rounded posterior extremity; claspers pale brown, not highly chitinised, broad with somewhat spoon-like ends. Harpes without posterior rod-like structures, dorsal root-like basal process of the side-pieces long and slender with a small barb at its posterior third, fused proximally with its fellow of the opposite side to form a wide arch, as figured by Carter, in the middle of which is a slight thickening. Aedoeagus delicately chitinised (excepting at the base, where it is stronger) especially laterally, tapering distally so as to appear conical in ventral view, with a small terminal lip, which is slightly everted ventrally.

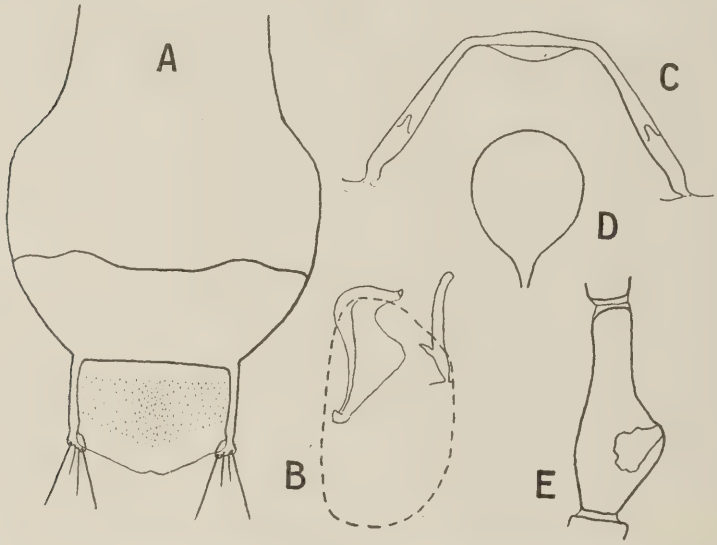


FIG. 15. *Forcipomyia ingrami*, Carter. *A*—Outline of ninth segment after removal of forceps and aedoeagus, ventral view. *B*—Hypopygium of ♂, lateral view, to show the relative positions of the aedoeagus and the dorsal process arising at the base of the side-piece. *C*—Dorsal processes arising from the bases of the side-pieces, ventral view of a disarticulated and slightly flattened specimen. *D*—Spermatheca of ♀. *Forcipomyia nigeriensis*, sp.n. ♀: *E*—Third segment of palp. *A*, *B*, *D*, and *E* × *c.* 265; *C* × *c.* 400).

GOLD COAST: Accra, abundant in the laboratory and in houses at all times of the year; Bole, 3.9.1911, 1 ♂ (Dr. A. Ingram); Kasunya, July, 1922, 1 ♂ (Dr. J. W. S. Macfie), reared from plants of *Pistia stratiotes* from the lagoon.

NIGERIA: Ilorin, 23.4.1912, 1 ♀ (Dr. J. W. S. Macfie); Yaba, near Lagos, 25.5.1909 and 28.6.1909, 2 ♀♀ (Dr. W. M. Graham), also 25.7.1913, 1 ♀ (Dr. J. W. S. Macfie); Calabar, February, 1922,

1 ♂ (Dr. E. C. Braithwaite); Victoria, April, 1921, 1 ♀ (Dr. L. H. Booth).

This species appears to resemble closely *F. rufescens* (Kieffer 1918), a Tunisian species, but to differ from it in general colouring, being usually dark brown with yellowish-brown legs, and not reddish with pale yellow legs. Moreover, *F. rufescens* is described as having the first and third veins of the wing fused in their proximal two-thirds, whereas in *F. ingrami*, Cart. (female) they are contiguous in their basal halves, but actually form a very narrow cell difficult to distinguish, and the scutellum yellow in the female and dark brown in the male, whereas in *F. ingrami* it is brown in both sexes. *F. seychelleana* (K.), *F. seychelleana*, var. *fulvithorax* (K.), and *F. kribiensis* (K.), also closely resemble *F. ingrami*; indeed, in the present state of knowledge it is difficult to select from the descriptions, satisfactory points of distinction, and it is possible some of the names are synonyms. An examination of the genitalia, which in Kieffer's species has not yet been made, would probably help in elucidating this matter.

### *FORCIPOMYIA EXIGUA*, sp.n.

Length of body (two females), 1·0 mm.; length of wing, 0·8 mm.; greatest breadth of wing, 0·3 mm. A small, brown species with a pale brown scutellum, three rather indistinct brown bands on the dorsum of the thorax, and large white halteres.

*Head* darkish brown, occiput densely clothed with rather long brown hairs. Eyes bare, attenuated and narrowly separated above, the facets about 25$\mu$ apart. Clypeus, proboscis, and palpi brown. Palpi (fig. 16, A) with the second segment small, only slightly longer than broad; fourth and fifth sub-equal, about twice as long as broad; third as long as the fourth and fifth together, inflated at the base and furnished with a sensory pit of moderate size. *Antennae* uniformly brown; torus rather darker than the flagellum segments. Segments 3 to 10 bearing stout, colourless spines, about twice as broad as the hairs, but not so long as the segments; and whorls of about twelve brown hairs. Flagellum segments forming an almost continuous series with no abrupt change of shape between the tenth and eleventh segments, but the distal segments rather longer and more attenuated

apically ; the fourth measuring in one specimen 8 by 7, and the fourteenth 11 by 6 units, the fifteenth segment broader than the others, about 14 units long, including the short terminal stylet. The combined length of segments 11 to 15 practically equal to that of segments 4 to 10, namely, about 60 units. *Thorax* brown ; dorsum darker brown than the pleura, with three broad longitudinal darkish brown stripes, the middle one deficient posteriorly and the lateral ones deficient anteriorly. Scutellum pale brown, bearing an irregular transverse row of about ten bristles, and about fourteen smaller hairs. Post-scutellum brown, about the same colour as the mesonotum. *Wings* unadorned, densely clothed with decumbent hairs, but devoid of scales. Microtrichia minute. Fringe as usual. Costa short, not reaching to the middle of the wing. First and third veins contiguous in their basal two-thirds, the first cell obsolete or indistinct, the second definite but small (fig. 16, B). Bifurcation

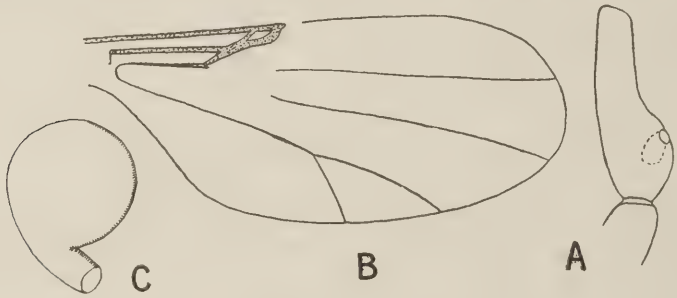


FIG. 16. *Forcipomyia exigua*, sp.n. ♀: *A*—Third segment of palp, *B*—diagram of wing venation, *C*—Spermatheca. *A* and *C* × *c.* 400, *B* × *c.* 80.

of the fifth vein a little distal to the level of the end of the costa. Halteres relatively large, with white knobs. *Legs* uniformly pale brown, bearing shortish hairs, and devoid of scales. First tarsal segment about two-and-a-half times the length of the second on all the legs ; on the hind legs about as long as the four succeeding segments. Claws and empodium as usual. *Abdomen* brown, tergites with broad darkish brown bands covering almost the entire surface ; sparsely clothed with hairs, but devoid of scales. Spermatheca (fig. 16, C) single, highly chitinised, shaped like a retort, the body sub-spherical, about 45$\mu$ in diameter, the chitinised portion leading to the commencement of the duct unusually broad, about 12$\mu$.

GOLD COAST: Accra, 21.1.1920, 2 ♀♀, and 4.2.1922, 1 ♀, taken in the evening upon the windows of the laboratory.

This species differs from all the other species of the genus which we have examined in having only a single spermatheca.

### *FORCIPOMYIA ASHANTII*, sp.n.

Length of body (one male), 1·6 mm.; length of wing, 1·2 mm.; greatest breadth of wing, 0·35 mm. A rather light brown species; without scales.

*Head* darkish brown. Eyes bare, the facets separated dorsally by a narrow line. Clypeus, proboscis, and palpi darkish brown. Palpi similar to those of *F. castanea*. *Antennae* rather pale brown, the plumes dark brown with paler brown tips. Torus very large, dark brown, without hairs. Flagellum segments similar in general characters to those of *F. castanea*, but the last three rather shorter; the lengths of the five terminal segments in one male were 13, 52, 23, 17, and 25 units respectively. The combined length of segments 12 to 15 about equal to that of segments 3 to 11. *Thorax*: dorsum uniformly light chestnut-brown with a slight greyish pruinescence, hairs scanty and short. Pleura much the same colour as the dorsum, not distinctly paler as in *F. castanea*. Scutellum about the same colour as the scutum, bearing fewer setae and hairs than in *F. castanea*, namely about 30. Post-scutellum slightly darker brown than the scutellum. *Wings* in some lights white and finely iridescent; with a distinct pale golden spot about the middle of the anterior border covering the distal radial cell. On each side of this pale spot is a patch of dark brown macrotrichia, the distal patch being especially large and distinct, and half-way between this dark patch and the tip of the wing, the wing surface is slightly infuscated. In addition to these markings there are a number of dark brown macrotrichia in the region of the bifurcation of the fifth vein, which stand out rather prominently. Macrotrichia rather scanty, mostly pale brown. Costa reaching to the middle of the wing, first and third veins fused in their basal halves, but forming a rather large distal cell, bifurcation of the fifth vein slightly distal to the level of the end of the costa. Halteres very pale brown, the knobs containing white pigment. *Legs* almost uniformly yellowish-brown,

but with a rather inconspicuous, slightly darker brown spot at the distal end of the hind femora, and, in one specimen, with a trace of a brown band on the hind tibiae a little below the knee. First tarsal segment of the fore legs about the same length as the second; those of the middle and hind legs shorter than the second, about three-quarters the length. Claws and empodium as in *F. castanea*. *Abdomen* pale brown, with darker brown bands on the segments which are broadest and most conspicuous on the ventral surface and on the posterior segments. Hypopygium brown, the side-pieces pale brown apically and darker brown basally.

HYPOPYGIUM (fig. 17). Ninth segment similar to that of *F. castanea*, but sternite notched in the middle line posteriorly, and the hairs on the sternite fewer, reduced in the middle line to a single

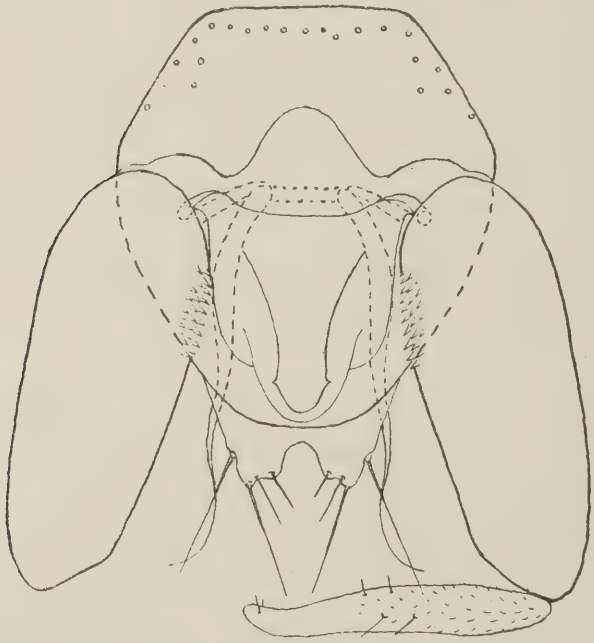

FIG. 17. *Forcipomyia ashantii*, sp.n. ♂: diagram of hypopygium, ventral view. × *c.* 400.

basal row. Forceps generally similar to those of *F. castanea*, but side-pieces rather long, and bearing on the inner basal aspect a patch of short, stout spines or teeth. Harpes united basally by a narrow

band of chitin, ending in long, slender filaments, resembling thus those of *F. biannulata*. Aedoeagus bluntly conical, rather long, not unlike that of *F. tigripes*.

GOLD COAST: Obuasi, Ashanti, April, 1920, 3 ♂♂ (Dr. A. S. Burgess); Nsawam, near Accra, 1 ♂, reared from vegetable matter taken from a rot-hole in a tree; Accra, 2 ♂♂, taken in the evening upon windows in the laboratory.

NIGERIA: Yaba, near Lagos, 23.5.1909, 1 ♂ (Dr. W. M. Graham), 'in bedroom at 9 p.m.'

This insect resembles in many respects *F. castanea*, but is a paler, more uniform, brown colour, the thorax not notably darker dorsally than laterally, and the hind femora with only an inconspicuous darker spot. The genitalia of the male are quite distinct from those of *F. castanea*.

### *FORCIPOMYIA MELANCHROA*, sp.n.

Length of body (one female), 3·0 mm.; length of wing, 1·6 mm.; greatest breadth of wing, 0·6 mm. A large, very dark brown, almost black, species; without scales.

*Head* very dark brown. Eyes bare; broadly contiguous above, the facets separated by a narrow line. Clypeus, proboscis, and palpi dark brown. Palpi similar to those of *F. castanea*: second, fourth, and fifth segments sub-equal, a little longer than broad; third about as long as the fourth and fifth together, its distal half narrow, its basal half greatly inflated and furnished with a large sensory pit. *Antennae* uniformly dark brown; torus rather small, scarcely darker than the flagellum segments. The large spines on segments 3 to 10 colourless or very pale brown, straight, pointed, as long as the segments, and at least twice as stout as the hairs; whorls of about twenty short, dark brown hairs. Segments 4 to 10 oval, sub-equal, the length and greatest breadth of the fourth and tenth being about 18 by 14, and 16 by 12 units respectively. Segments 11 to 15 elongated, sub-cylindrical; 11 to 14 sub-equal, about one-third longer than the tenth, and from two to three times as long as their basal breadth; the fifteenth slightly longer, about four times as long as broad, ending in a small blunt stylet. The combined length of segments 11 to 15 equal to that of segments 4 to 10, namely about

122 units. *Thorax* very dark brown, almost black. Dorsum showing indistinctly (because the insect is so dark), the three broad longitudinal bands common in this genus, and also a small short lateral band on each side. Pleura dark brown. Scutellum and post-scutellum almost black; the former bearing numerous bristles and hairs. *Wings* brownish, unadorned, darkest about the middle near the anterior border, densely clothed with dark brown decumbent hairs, but devoid of scales. Costa reaching a little beyond the middle of the wing. First and third veins darkish brown, fused in their proximal halves, but forming a distinct distal cell. Cross-vein and petiole of the fourth vein about equal. Bifurcation of the fifth vein just proximal to the level of the end of the costa. Halteres with pale brown knobs. *Legs* uniformly very dark brown, densely clothed with shortish, very dark brown hairs, devoid of scales. First tarsal segment about one-quarter longer than the second on the fore legs, and practically equal to the second on the middle and hind legs. Claws and empodium as usual. *Abdomen* uniformly very dark brown, well-clothed with shortish, very dark brown hairs, but devoid of scales. Spermathecae two, highly chitinised, oval, large, unequal or sub-equal, about 200$\mu$ to 300$\mu$ by 120$\mu$ to 150$\mu$; practically no part of the duct chitinised.

GOLD COAST: Accra, 26.12.1920, 1 ♀, taken in the evening in a bungalow; Obuasi, 23.11.1906, and 5.7.1907, 2 ♀♀ (Dr. W. M. Graham).

### *FORCIPOMYIA NIGERIENSIS*, sp.n.

Length of body (one female), 2·3 mm.; length of wing, 1·5 mm.; greatest breadth of wing, 0·5 mm. A large, dull brown species with some general resemblance to *F. inornatipennis* (Aust.), but with wings not so hairy, and legs a dull brown in place of a yellow brown. General colouring similar to that of *F. ingrami*. Without scales.

*Head* darkish brown. Eyes bare, contiguous above but rather narrowly, the facets separated by a narrow line. Clypeus, proboscis, and palpi darkish brown. Palpi (fig. 15, E) with second, fourth, and fifth segments sub-equal, not twice as long as broad; third segment as long as the fourth and fifth together, ham-shaped, the basal half much inflated and furnished with a large sensory pit which

has a small, circular, anterior opening. *Antennae* uniformly darkish brown. Torus about the same colour as the flagellum segments, rather small, bearing about a dozen hairs. Segments 3 to 10 bearing large spines, about twice as broad as the hairs, not quite as long as the segments, and with rather blunt ends; and whorls of about twelve dark brown hairs which are mostly only slightly longer than the segments. Flagellum segments forming an almost continuous series with no abrupt change of shape between the tenth and the eleventh segments, but the terminal segments rather longer and more constricted apically, the fourth measuring 16 by 12, and the fourteenth 19 by 10 units. Last segment unfortunately missing. So far as can be judged, however, the combined length of segments 11 to 15 would be rather less than that of segments 4 to 10, or 3 to 10, namely, about 94 as compared with 108 or 126 units respectively. *Thorax* uniformly darkish brown, clothed with short, slender, brown hairs; pleura not quite so deep a brown. Scutellum and post-scutellum darkish brown, the former bearing about twenty bristles and a few small hairs. *Wings* light brown, unadorned, well-clothed with rather slender decumbent hairs, but apparently devoid of scales. Costa extending slightly beyond the middle of the wing (46 : 81 units). First radial cell obsolete, second well-formed. Bifurcation of the fifth vein a little proximal to the level of the end of the costa. Halteres pale brown. *Legs* almost uniformly darkish brown, hairs rather short, devoid of scales. First and second tarsal segments sub-equal on all the legs, first actually very slightly longer. Claws and empodium similar to those of *F. castanea*. *Abdomen* brown, venter slightly paler than the dorsum, clothed with shortish hairs. Spermathecae two, not very highly chitinised, oval, sub-equal, large, about 150$\mu$ by 100$\mu$; the commencement of the duct chitinised for only a very short distance (about 7$\mu$).

NIGERIA: Lagos, 2.7.1921, 1 ♀ (Dr. H. A. Foy.)

# CHANGES IN THE BLOOD IN PRIMARY MALARIA

BY

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

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The observations that follow were made in England on three patients suffering from general paralysis of the insane, all of whom were undergoing treatment by a first attack of malaria, induced as the result of the bites of anophelines infected with *Plasmodium vivax* ; in these cases, therefore, it was possible to observe the blood changes that occurred in a patient suffering from an undoubted primary attack of malaria, the date of acquirement of which was known. So far as the writer is aware, all previously recorded examinations have reference to patients who may—or may not—have been suffering from a primary attack and whose exact date of infection was not known. Ross (1924) has drawn attention to the importance of such observations in cases of primary malaria ; the present work had already been started when his letter was published, but an attempt has been made to investigate one of the problems suggested by him ; that is to say, whether or not there is any noticeable reduction in the total number of red cells before the appearance of parasites in the peripheral blood.

The following were investigated :—

(1) *The total number of red cells per cubic millimetre of blood.* The counts were made in the usual manner with a Thoma-Zeiss haemocytometer, the second drop of blood from the finger being always examined ; it will be noted that the results are expressed in the table to the nearest half-million ; this is due to the fact that a previous series of counts on a normal individual appeared to show that half-a-million was the usual limit of working error.

(2) *The colour index.* A measured volume of the patients' blood was haemolysed in distilled water and compared with the same

volume of a normal individual's blood also in distilled water (the same control being used throughout) ; the percentage of haemoglobin was then compared with the red cell count taken at the same hour, and the result expressed as the colour index.

(3) *Ratio of infected to non-infected red cells.* An adjustable counting eye-piece was used and set to cover a field of forty erythrocytes ; one thousand two hundred and fifty such fields were examined and the number of infected red cells in each field noted. It was found that in an evenly-spread film the number of red cells in such relatively small fields remained very constant, as was shown by counting the actual number of red cells present in every thirtieth field and if necessary, re-adjusting the eye-piece.

As already stated, previous writers' figures are concerned with the blood changes occurring in patients whose incubation period is unknown and whose previous history as regards malaria is uncertain ; it seems unprofitable, therefore, to compare these figures with those considered in the present paper. It may, however, be mentioned that Mannaberg (1894) quotes a large number of figures, both from his own observations and from those of other writers, which show that the reduction in the red cells varies greatly in different cases, but that the haemoglobin reduction is almost always directly proportional to the fall in the erythrocyte count ; that is to say, it is of the simple anaemia type. More comparable figures may be had from animal experiments ; thus Ben-Harel (1923) infected twenty-three birds by intramuscular injection of *Proteosoma praecox ;* he divides the resultant malaria into three types, primary acute infections, extended irregular infections, benign infections. Amongst other observations he clearly demonstrates, by means of daily blood counts, that : (1) In all three types a definite reduction in the total number of red cells occurred during the incubation period ; (2) there was no further fall in the number of red cells after the disappearance of parasites from the circulation ; (3) in all cases in which the infection did not prove fatal, the parasites increased to a certain maximum concentration and then proceeded to fall in numbers at approximately the same rate as that at which they had previously increased. This fall was sometimes interrupted by a relapse, causing a sharp temporary rise.

As the present writer only observed three cases, it would be

TABLE I.—Showing the results of blood examinations in three cases of primary malaria.

CASE 1. Male. Aged 38.

| Day of observation— 1 = day of infection. 2 = day after infection, etc. | 1 | 4 | 7 | 9 | 12 | 13 | 14 | 16 | 18 | 20 | 22 | 23 | 24 | 26 | 28 | 30 | 32 | 34 | 36 | 38 | 51 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of red cells per c.mm., in millions, to the nearest half-million ... ... ... | 5½ | 5½ | 5½ | 5½ | 5½ | ... | 5½ | 5½ | 5 | 4½ | 4½ | ... | 3½ | 4 | 4 | 4 | 4 | 4 | 4 | 4½ | 4½ |
| Total number infected amongst 50,000 red cells examined ... ... ... ... ... | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 62 | 217 | 172 | 180 | ... | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Colour index ... ... ... ... ... ... | 1 | 1 | 1 | 1 | 1 | ... | 1 | 1 | 0.9 | 0.9 | 0.9 | ... | 1 | 1 | 1 | 1 | 0.9 | 0.9 | ... | 0.9 | 1 |
| Quinine ... ... ... ... ... ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 grs. | 30 grs. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

CASE 2. Female. Aged 13.

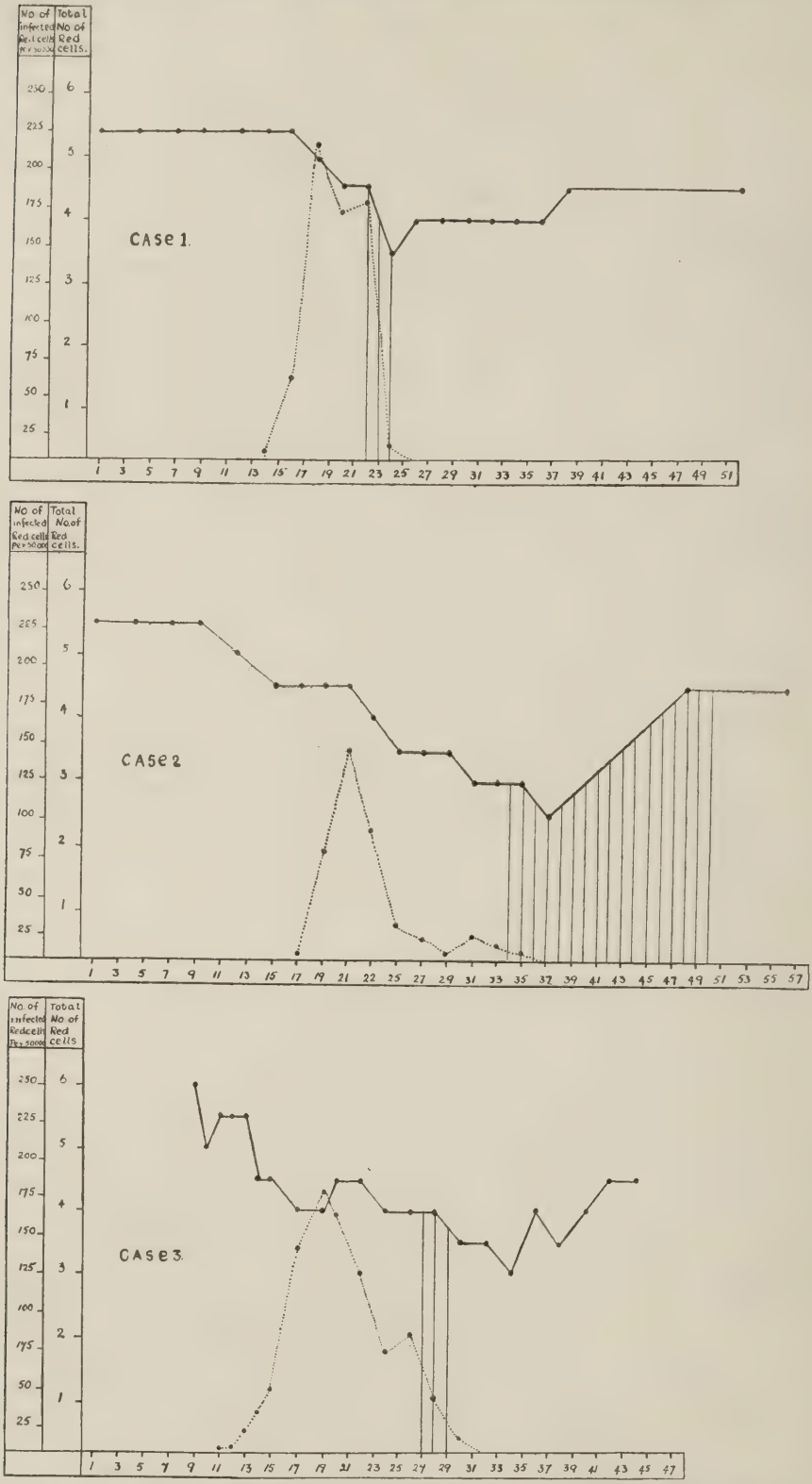
| Day of observation— 1 = day of infection. 2 = day after infection, etc. | 1 | 4 | 7 | 9 | 12 | 15 | 17 | 19 | 21 | 23 | 25 | 27 | 29 | 31 | 33 | 35 | 37 | 48 | 56 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of red cells per c.mm., in millions, to the nearest half-million ... ... ... ... ... ... ... | 5½ | 5½ | 5½ | 5½ | 5 | 4½ | 4½ | 4½ | 4½ | 4 | 3½ | 3½ | 3½ | 3 | 3 | 3 | 2½ | 4½ | 4½ |
| Total number infected amongst 50,000 red cells examined ... | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 80 | 146 | 94 | 33 | 24 | 8 | 25 | 14 | 7 | 0 | 0 | 0 |
| Colour index ... ... ... ... ... ... ... | 0.9 | 0.9 | 0.9 | 0.95 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.85 | 0.85 | 0.8 | 0.8 | 0.8 | 0.8 | 0.9 | 0.85 | 0.9 |
| Quinine ... ... ... ... ... ... ... ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Quinine grs. 10 daily, from the 35th to the 50th day, inclusive. | | | 0 |

CASE 3. Male. Aged 44.

| Day of observation— 1 = day of infection. 2 = day after infection, etc. | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 17 | 19 | 20 | 22 | 24 | 26 | 28 | 29 | 30 | 32 | 34 | 36 | 38 | 40 | 42 | 44 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of red cells per c.mm., in millions, to the nearest half-million... | 6 | 5 | 5½ | 5½ | 5½ | 4½ | 4½ | 4 | 4 | 4½ | 4½ | 4 | 4 | 4 | ... | 3½ | 3½ | 3 | 4 | 3½ | 4 | 4½ | 4½ |
| Total number infected amongst 50,000 red cells examined ... ... ... | 0 | 0 | 2 | 4 | 23 | 30 | 51 | 143 | 178 | 165 | 125 | 75 | 85 | 43 | ... | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Colour index ... ... ... ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Quinine ... ... ... ... ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 grs. | 30 grs. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE II

Graphical records showing the results of blood examinations in three cases of primary malaria.




*Explanation of Table.* The dotted line represents the total number of infected red cells amongst fifty thousand examined. The continuous horizontal line represents the total number of red cells expressed in millions to the nearest half million. The vertical lines delimit days on which quinine was given, in cases one and three the amount given was thirty grains daily, in case two, ten grains daily. The base line shows the days on which observations were recorded : 1 being the day of infection, 2 the day after infection, etc.

unwise to draw any definite conclusions, but the following points appear to be of interest, especially when compared with Ben-Harel's corresponding figures for birds. (1) In case No. 2 there was a marked diminution in the number of red cells for several days previous to the first appearance of parasites, and the same, though in a less marked degree, is true of No. 3 case; on this particular point, therefore, these two cases accord with Ben-Harel's findings. (2) In at least one patient, the red cell count continued to fall after the disappearance of parasites from the peripheral circulation. This is contrary to all Ben-Harel's findings. (3) The parasites, after reaching a maximum concentration, on or about the fifth day after their first appearance, tended to diminish in numbers; this diminution was slower than the previous increase and took place whether quinine was given or not; it was clearly observable in all three cases, but most marked in cases two and three, in which the maximum concentration was in each case reached somewhere between fourteen and twenty thousand parasites to the cubic millimetre. On the whole, these figures correspond closely with those given by Ben-Harel under the title 'Acute primary infection,' although in some of his cases the parasitic concentration reached was far higher. (4) In two cases where the colour index was estimated it was found to be of the simple anaemia type.

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# NOTES ON SOME NEMATODES IN THE MUSEUM OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

E. L. TAYLOR, M.R.C.V.S.

(*Received for publication* 10 *November*, 1924)

### *PORROCAECUM CROCODILI*, n sp.

Host :—Crocodile. Position :—Stomach. Locality :—Gold Coast.

This worm is of medium size (6·7 by 0·25 mm. to 32 by 1 mm.). The body tapers gradually towards the head, and terminates abruptly at the posterior extremity ; it is marked by fine transverse striations and a few scattered, minute papillae ; at a distance of about 0·7 mm. from the anterior extremity are two small cervical papillae, placed laterally and just posterior to the nerve ring. The excretory pore is large and somewhat resembles a papilla ; it is, as is usual in this genus, situated between the bases of the two subventral lips. The three lips forming the head (figs. 1 and 2) are 0·13 to 0·18 mm. in diameter, and a little more than half that height ; they present a semi-lunar outline when viewed laterally, and two antero-internal lobules are seen standing above the semicircular anterior margin of the lips ; each of these lobules carries eight or ten denticulations, the rest of the lips is not armed in this way. The dorsal lip carries two long papillae, notched about their middle, while the two subventral lips only have one papilla each, carried towards the ventral side of the centre of the lips. Viewed anteriorly, the lips are seen to have a notch about the middle of their internal aspect, which might be said to divide the anterior lobe of the lip into two. Each lip is marked off from the body by a definite line round its base. The oesophagus is long and slender, measuring up to 4·87 mm. in length (about one-seventh of the total length of the worm), its diameter is uniform throughout the whole length ; a straight, oblong, oeso-

phageal ventriculus is present, measuring about 0·675 mm. long, and there is a well-developed intestinal caecum, extending forward from the end of the intestine to a point slightly anterior to the middle of the oesophagus.

The male measures 8·00 to 24·75 mm. in length and 0·45 to 0·80 mm. in its greatest diameter. The two spicules are of equal

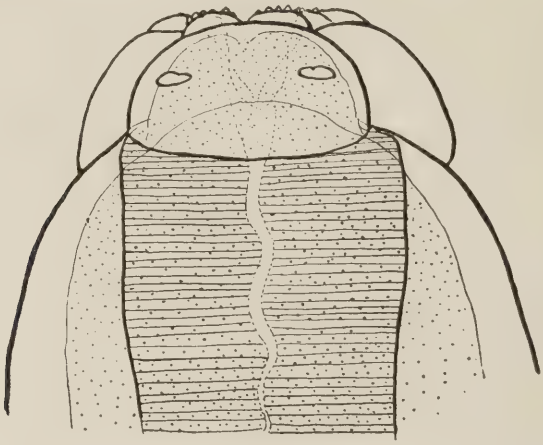

FIG. 1. *Porrocaecum crocodili*, n.sp. Head, dorsal view. × 253.

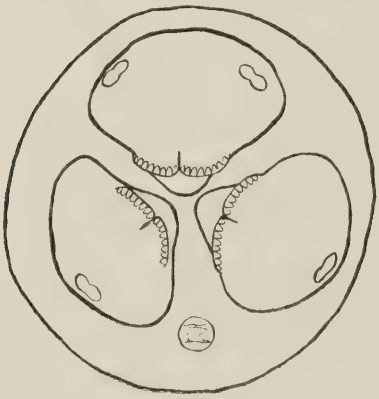

FIG. 2. *Porrocaecum crocodili*, n.sp. Head, anterior view. × 190.

length, tubular in form, not alate, and measuring 0·58 to 0·78 mm. in length. A small but well-defined gubernaculum is present, 0·075 to 0·08 mm. long ; caudal alae are absent. The caudal papillae are in two groups (figs. 3 and 4) : firstly a long file of 30 to 37 pedunculated

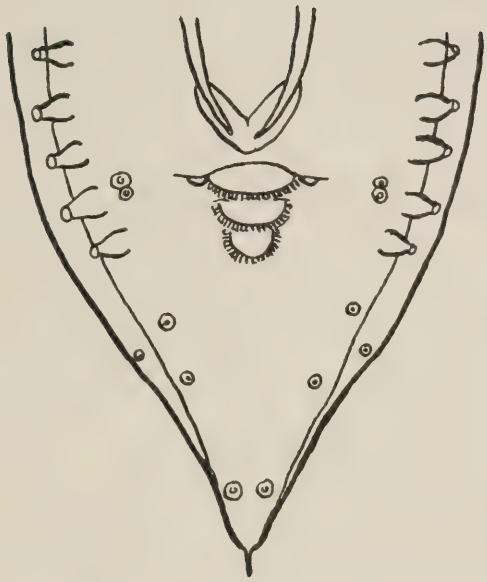

Fig. 3. *Porrocaecum crocodili*, n.sp. Caudal extremity of male, ventral view. × 160.

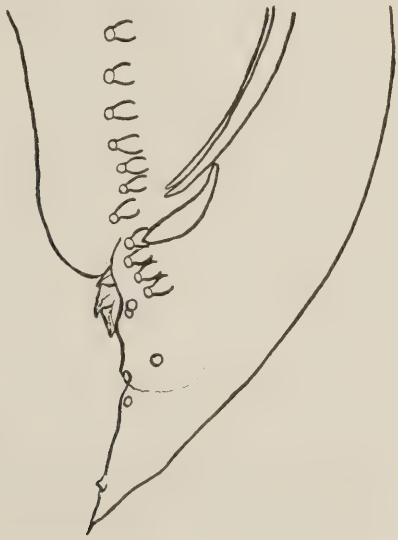

Fig. 4. *Porrocaecum crocodili*, n.sp. Caudal extremity of male, lateral view. × 260.

papillae on either ventro-lateral aspect, the posterior three or four pairs being postanal; and secondly, six pairs of sessile papillae: one ventral, level with the cloaca, a double papilla subventral at the same level, a group of three subventral, forming a triangle half-way between the cloaca and extremity of the tail, and one larger pair placed ventrally further back. Immediately behind the cloaca are three curious semicircular projections from the cuticle; these are directed backwards and have very thin, serrated, free edges; viewed laterally, they look like sharp spines directed backwards from the cloaca. The extremity of the conical tail is an extremely acute point; the distance from the tip to the cloaca is about 0·18 mm.

The female measures 6·7 to 32 mm. in length and 0·25 to 1 mm. in greatest diameter; the vulva is placed just in front of the middle of the body; from here the vagina passes backwards to where it finally divides into the two branches of the uterus. Behind the anus the tail rapidly tapers off to end in a short, sharp point; the length of the extremity beyond the anus is about 0·21 mm.

It must be noted that the specimens available were not gravid.

### *AMPLICAECUM AFRICANUM*, n.sp.

Host :—*Bufo regularis*. Position :—Stomach. Also found in the stomach of a crocodile along with the bones of a frog or toad. Locality :—West Coast of Africa.

This worm is of medium size, measuring 22 to 50 mm. in length and up to as much as 1·2 mm. in thickness. The body gradually tapers towards the head, but terminates abruptly behind in an obtuse cone, which, in the female, is remarkably short. The cuticle is thick and marked with fine transverse striations; the posterior extremity is usually coiled. The excretory pore is situated at about 0·75 mm. from the head, while at a slightly higher level is the nerve ring and two small, sessile cervical papillae. A dorsal papilla may be present, situated about 1·5 mm. from the head. The three lips (fig. 5) are more or less rectangular and measure about 0·27 mm. in diameter; each presents two grooves on its inner aspect, one parallel to the long axis of the body, and one transversely; denticulations are easily seen, and follow the margins of the lips throughout their whole extent. The dorsal lip carries two large papillae, and each

ventro-lateral lip one large and one small papilla ; the large papilla, as usual, being placed towards the ventral side of the lip, and the small one laterally and a little more anteriorly. The large papillae present the peculiar appearance of having a segment cut out of their

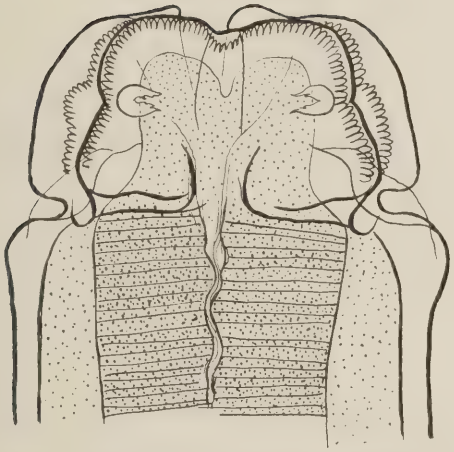

FIG. 5. *Amplicaecum africanum*, n.sp. Head, dorsal view. × 190.

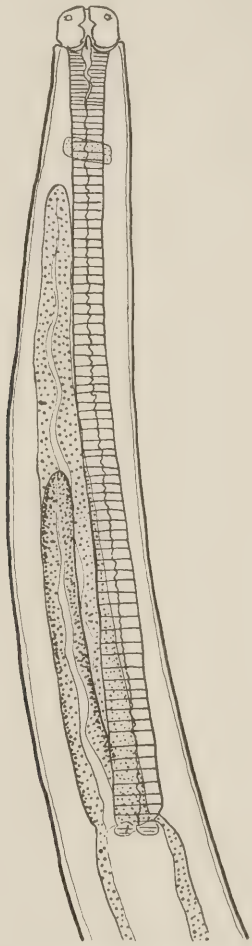

FIG. 6. *Amplicaecum africanum*, n.sp. Oesophageal portion, showing two intestinal caeca. × 40.

inner aspect. The pulp is divided into two equal lobes with even, rounded ends ; small interlabia are present, from which well-marked grooves encircle the bases of the lips. The oesophagus is 3·0 to 4·5 mm. in length (about one-eleventh of the length of the worm)

and about 0·27 mm. wide, throughout its whole length, excepting for a little widening at its two extremities. One large intestinal caecum is present—length about 3·57 mm.—and just where it leaves the intestine it measures about 0·42 mm. in diameter, becoming narrower towards the anterior extremity, and terminating about 0·9 mm. from the head. Sometimes a second caecum is found to be present, and when this occurs, it is only half the length of the one normally seen (fig. 6).

The male measures from 22 to 30 mm. in length and 0·46 to 0·68 mm. in its greatest width; the width at the anus is about 0·35 mm. and the length of the caudal extremity behind the anus about 0·31 mm. Well-developed caudal alae are present, into the

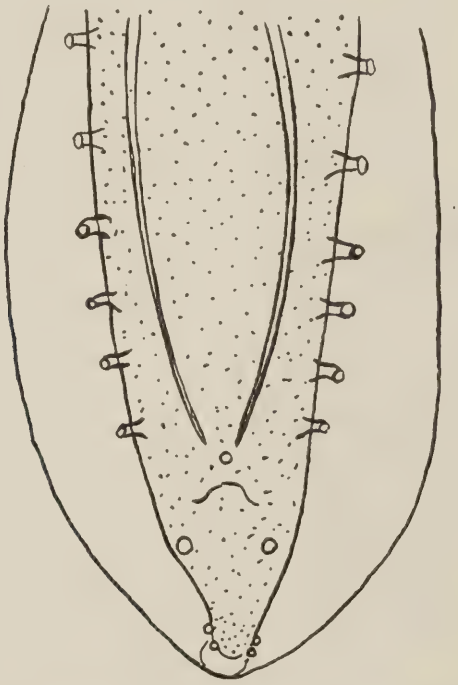

Fig. 7. *Amplicaecum africanum*, n.sp. Caudal extremity of male, ventral view. × 110.

substance of which the pedunculated preanal papillae run (figs. 7 and 8); these papillae are 10 to 12 in number, the anterior members being sessile and small. Postanal papillae are three in number on either side, two very small pedunculated papillae placed laterally

near the extremity, and one larger, sessile papilla placed sub-ventrally just behind the cloaca; there is one unpaired median papilla on the elevation in front of the anus. The spicules are equal and measure about 0·85 mm. in length and 0·013 mm. in diameter; they have a sharp barb at their extremities. Gubernaculum is absent.

The female measures 32 to 50 mm. in length and 0·66 and 1·2 mm. in width; the vulva is situated anterior to the middle, the vagina can be traced backwards from here, and the coils of the ovaries are placed in the posterior part of the body up to 1·5 to 2 mm. from the caudal extremity. The caudal extremity is blunt, extending for about 0·3 mm. behind the anus; it carries a small pointed button at

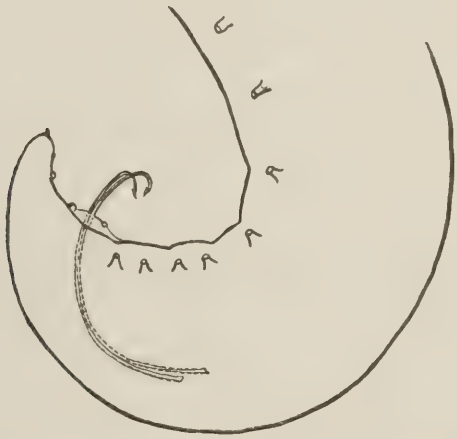

Fig. 8. *Amplicaecum africanum*, n.sp. Caudal extremity of male, lateral view. × 55.

the tip; the width at the anus is 0·63 mm. The eggs are oval and large, 135 by 83$\mu$; the shell is thin and opaque, being marked with numerous small depressions on its surface.

This worm shows considerable similarity with *Amplicaecum colurum* from the eagle (Baylis, 1919), but in the latter species the oesophagus is about one-seventh the complete length of the worm, while the papillae on the dorsal lips are absent. Unfortunately, Baylis had only two female specimens, so that it is impossible to say how closely the species here described resembles *A. colurum*: the identity of the species does not, however, seem probable in view of their two widely different hosts.

On comparison with *Amplicaecum varani*, the only other known species of this genus, the differences are again found to be slight, but, apart from mere difference in size, the intestinal caecum measures one-thirteenth of the body length in the species here described, against only one-twenty-fourth in *A. varani*; and the caudal papillae of the male are fewer in number, being fourteen pairs of preanal papillae, against thirty-two in *A. varani*, and three pairs of postanal against five in *A. varani*.

It is interesting to note the presence in some individuals of two intestinal caeca, and it appears that this is a character in which some individual variation may be expected to occur. Baylis (1921) has drawn attention to a somewhat similar variation in *Polydelphis quadricornis* and (1922) in *P. sewelli*, in which two species only some individuals showed the presence of this intestinal appendage.

### *STRONGYLURIS BREVICAUDATA*, Müller, 1894

Host :—*Agama colonorum*. Position :—Intestine. Locality :—Northern Nigeria.

Body tapers gradually from the middle to the head, and measures 5·5 to 12 mm. in length. The cuticle is thick and marked with fine transverse striations and carries numerous minute papillae arranged at fairly regular intervals in longitudinal lines ; the intervals between the papillae increase towards the posterior extremity. These papillae are much more clearly seen in some specimens than in others, but I have not seen any special crown of papillae below the head. The head (fig. 9) is formed by three lips and is narrower than the neck immediately behind, so that a ' shoulder ' is formed. Each of the three lips carries a pair of papillae, while at the anterior edge is a very delicate membrane which can only be seen when the lips are extended forward ; in specimens where the lips are closed together this membrane is out of sight, but seen on edge when extended, it gives to the lip a hooked appearance. Behind the mouth is a well-developed pharynx (fig. 10) 0·18 to 0·23 mm. in length ; at its junction with the second part of the oesophagus is a small cavity in the form of a kink in the lumen of the tube. The second portion of the oesophagus usually describes one or two wide curves before reaching the bulb at the posterior end ; the length of the entire

oesophagus is 1·8 to 2·4 mm., from 1/5·2 to 1/3 of the length of the whole worm. The nerve ring is placed about twice the length of the pharynx from the head. The intestine, at its junction with the oesophagus, is considerably wider than the oesophageal bulb.

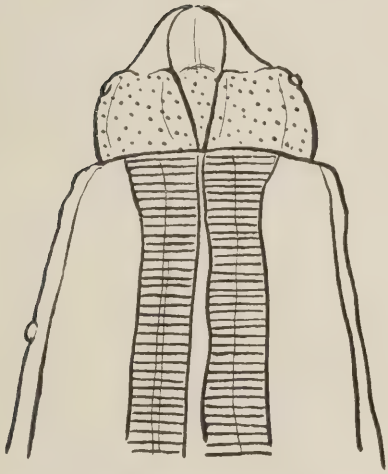

Fig. 9. *Strongyluris brevicaudata.* Anterior extremity. × 333.

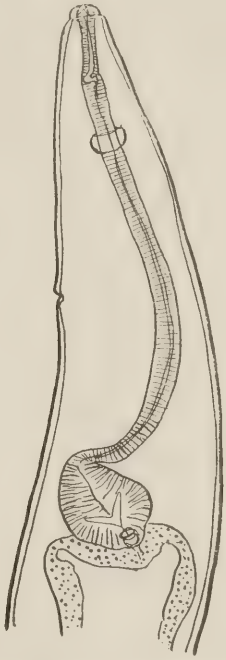

Fig. 10. *Strongyluris brevicaudata.* Oesophageal portion. × 40.

The male measures 5·5 to 9·6 mm. in length and 0·33 to 0·6 mm. in its widest part. The caudal alae (fig. 11) are wide and short, and

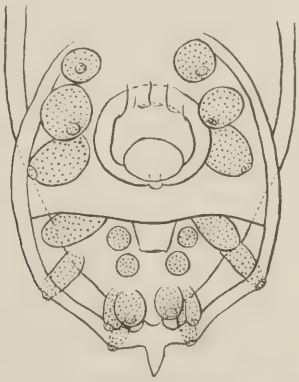

Fig. 11. *Strongyluris brevicaudata.* Caudal extremity of male, ventral view. × 125.

have thickened margins which almost meet on the ventral surface in front of the preanal sucker. The papillae number ten pairs. Of these, three are placed at the side of the preanal sucker, the anterior one is often small and sometimes absent, while the middle and posterior ones are large flask-shaped papillae. Of the remaining seven pairs, two sessile pairs are placed ventrally at the side of the anus, while two pedunculated pairs are placed lateral to these, the other three pairs of papillae being grouped on either side of the extremity. Some specimens show one or two very small, sessile papillae subventrally placed in front of the anus. The length of the caudal extremity behind the cloaca is 0·07 to 0·11 mm. (1/105 to 1/75 of the length of the whole worm) ; it has a small spike at the extreme end. The preanal sucker has a strongly chitinised rim and is circular in outline, measuring 0·12 mm. in diameter. The spicules are in the form of long, tapering tubes of equal measurement from 1·5 to 1·95 mm. (1/5·6 to 1/3·6 of the body length) and have a reticulated surface.

The female measures 6·75 to 12 mm. in length and 0·345 to 0·855 mm. across the widest part ; the vulva is situated posterior to the middle and takes the form of a transverse slit with salient lips. The vagina describes a very winding course ; after first taking a forward direction for about 0·42 mm., it bends backwards, dividing into the two branches of the uterus ; these continue to a point about 1·2 mm. from the caudal extremity, where they bend forward again to reach the ovaries, which are situated in the anterior part of the body. The caudal extremity of the female is in the form of a short cone 0·12 to 0·21 mm. long from anus to extremity and carries two small papillae. The eggs are oval, about $73\mu$ by $43\mu$ in size, unsegmented, and with a thick, smooth shell.

Details of measurement of closely allied members of the genus *Strongyluris* are compiled in Table I. The morphological characters of these seven species, as described by the various observers, are practically identical ; *S. gigas* is the only species showing marked variation in detailed measurements from the one described above, while *S. calotis*, *S. ornata* (Gendre) and *S. ornata* (Linst.) only show variation in the ratio of spicule length to body length. It seems more than likely that the strongylurids described under the specific names *chamaelonis*, *streptoesophagus* and *brevicaudata* are one and the

TABLE I. Details of measurements of allied species of *Strongyluris*.

| Name | *S. brevicaudata*, Müller [Spaul, 1923] | | *S. brevicaudata*, Müller here described | | *S. streptoesophagus*, Connall, 1912 | | *S. chamaelonis* Baylis & Daubney, 1922 | | *S. calotis* Baylis & Daubney, 1923 | | *S. ornata* (Gendre) [Spaul, 1923] | | *S. ornata* (Linst.) [Spaul, 1923] | | *S. gigas*, Spaul, 1923 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Host | *Agama*, sp. | | *Agama colonorum* | | *Agama colonorum* | | *Chamaelon vulgaris* | | *Calotis nigrilabris* | | *Agama colonorum* | | *Stello vulgaris* | | *Agama distanti* | |
| Locality | East Africa | | West Africa | | West Africa | | Calcutta | | Ceylon | | Africa | | Madagascar | | East Africa. | |
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Length | 9.6 | 10.5 | 5.5 – 9.6 | 6.75 – 12 | 8.6 | 9.1 | 6.3 | 8.4 – 8.75 | 8.9 – 11.1 | 11 – 13.65 | 12 – 15 | 13 – 17 | 12 | ... | 32 | 35 |
| Breadth | 0.45 | 0.55 | 0.33 – 0.6 | 0.345 – 0.855 | 0.44 | 0.5 | 0.5 – 0.7 | | 0.4 – 0.5 | 0.55–0.75 | 0.76–0.84 | 0.9 – 1 | 0.83 | ... | 1.2 – 1.5 | 1.5 |
| Length of tail | 0.1 | 0.2 | 0.07 – 0.11 | 0.12 – 0.21 | 0.01 | 0.2 | 0.13 | 0.3 | 0.1–0.12 | 0.2 – 0.25 | ... | ... | ... | ... | 0.25 | 0.4 |
| Relative length of tail | $\frac{1}{95}$ | $\frac{1}{50}$ | $\frac{1}{105} - \frac{1}{71}$ | $\frac{1}{85} - \frac{1}{40}$ | $\frac{1}{860}$ | $\frac{1}{45}$ | $\frac{1}{48}$ | $\frac{1}{29}$ | $\frac{1}{90} - \frac{1}{89}$ | $\frac{1}{55} - \frac{1}{54}$ | $\frac{1}{93} - \frac{1}{90}$ | $\frac{1}{67} - \frac{1}{61}$ | $\frac{1}{100}$ | $\frac{1}{60}$ | $\frac{1}{125}$ | $\frac{1}{90}$ |
| Length of pharynx | 0.25 | 0.29 | 0.18 – 0.22 | 0.20 – 0.23 | 0.23 | ... | 0.18 – 0.22 | | 0.26 – 0.3 | ... | ... | ... | ... | ... | 0.3 | 0.4 |
| Length of oesophagus | 1.9 | 2 | 1.8 – 2.17 | 1.8 – 2.4 | 1.58 | 1.95 | 1.1 | 1.45 | 1.75 – 2.25 | | ... | ... | ... | ... | 3 | 3.5 |
| Relative length of oesophagus | $\frac{1}{5.1}$ | $\frac{1}{5.25}$ | $\frac{1}{4.9} - \frac{1}{3}$ | $\frac{1}{5.2} - \frac{1}{3.6}$ | $\frac{1}{5.4}$ | $\frac{1}{4.6}$ | $\frac{1}{5.7}$ | $\frac{1}{6.0}$ | $\frac{1}{6.3}$ | $\frac{1}{6}$ | $\frac{1}{5.6} - \frac{1}{4.7}$ | $\frac{1}{5.3} - \frac{1}{5}$ | $\frac{1}{6}$ | ... | $\frac{1}{12} - \frac{1}{10}$ | $\frac{1}{10} - \frac{1}{9}$ |
| Length of spicules | 1.3 | ... | 1.5 – 1.95 | ... | 1.2 | ... | 1.1 | ... | 0.75 – 0.8 | ... | 1.5 | ... | 1.06 | ... | 1.1 | ... |
| Relative length of spicules | $\frac{1}{7.4}$ | ... | $\frac{1}{5.6} - \frac{1}{3.6}$ | ... | $\frac{1}{7}$ | ... | $\frac{1}{5.7}$ | ... | $\frac{1}{14}$ | ... | $\frac{1}{10}$ | ... | $\frac{1}{11}$ | ... | $\frac{1}{29}$ | ... |
| Width of oesophagus | 0.04 | 0.07 | 0.043 – 0.068 | 0.06 – 0.085 | 0.08 | 0.07 | ... | ... | ... | ... | ... | ... | ... | ... | 0.085 | 0.096 |
| Vulva from post. ext. | ... | 3.5 | ... | 3.75 – 6.75 | ... | 3.35 | ... | 3 – 3.3 | ... | 4.7 – 5.65 | ... | ... | ... | ... | ... | 17 |
| Ratio of above measurement to body length | ... | $\frac{10}{30}$ | ... | $\frac{10}{22} - \frac{10}{27}$ | ... | $\frac{10}{27}$ | ... | $\frac{10}{26}$ | ... | $\frac{10}{24}$ | ... | $\frac{10}{23}$ | ... | $\frac{10}{20}$ | ... | $\frac{10}{21}$ |
| Sucker from post. ext. | ... | ... | 0.2 | ... | 0.76 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.4 | ... |
| Diameter of head | ... | ... | 0.07 – 0.08 | | ... | ... | ... | ... | 0.06 – 0.08 | | ... | ... | ... | ... | ... | ... |
| Eggs, length | ... | 63μ | ... | 73μ | ... | 60μ | ... | 87.5μ | ... | 87.5μ – 97.5μ | ... | 76μ | ... | 70μ | ... | 150μ |
| Eggs, breadth | ... | 48μ | ... | 43μ | ... | 30μ | ... | 55μ | ... | 50μ | ... | 44μ | ... | 47μ | ... | 90μ |

All the above figures are in mm. unless otherwise stated.

same species, while it is not improbable that the species *calotis*, *ornata* (Gendre) and *ornata* (Linst.) will also prove to be identical with the genotype *S. brevicaudata*.

### *AFRICANA AFRICANA* (Gendre)

Host :—Lizard (species of skink) and *Bufo regularis*. Locality :—Northern Nigeria.

This worm is of small size, 3·17 to 5·77 mm. long ; the cuticle, which is thin, is marked with fine cross striations ; longitudinal lines in the musculature show clearly through ; there are two narrow lateral flanges 19 to 29$\mu$ in width which terminate a short distance from either extremity ; a few minute papillae may be seen on the body surface. The mouth is surrounded by three subglobular lips (fig. 12), each bearing a pair of papillae, and separated

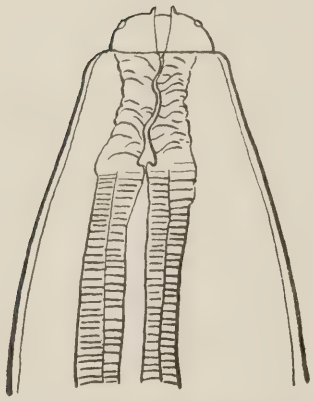

Fig. 12. *Africana africana.* Anterior extremity. × 253.

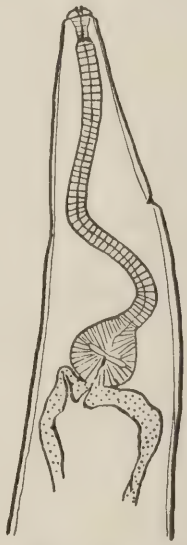

Fig. 13. *Africana africana.* Oesophageal portion. × 53.

from the neck by a well-marked line ; the diameter of the head is 50 to 68$\mu$ and its depth 26 to 29$\mu$. The excretory pore is situated about half-way down the oesophageal portion of the body. Following the small infundibuliform mouth cavity is a short pharynx 46 to 60$\mu$ in length, which widens posteriorly to form a small cavity, but the

ventral kink seen so plainly in *Strongyluris* is not present. The oesophagus (fig. 13) 1·05 to 1·2 mm. in its entire length, usually describes two curves between the pharynx and the bulb ; this latter structure is well-developed and measures about 0·200 by 0·180 mm. ; the intestine at its junction with the oesophagus is wider than the bulb.

Male, 4·5 to 4·95 mm. in length and 0·321 to 0·37 mm. at its widest measurement ; it has two slightly unequal spicules varying in length from 1·55 to 1·73 mm. and up to 0·846 mm. in breadth at their widest part ; there is a ventral notch at the proximal end, and each spicule presents two ventral gutters running parallel throughout the greater part of its length to a point near the apex ; the surface of the spicules is reticulated. Caudal alae are present, apart from the lateral flanges, and measure about 0·075 mm. at their widest part, which is about the level of the preanal sucker. The tail (figs. 14 and 15) is

FIG. 14. *Africana africana.* Caudal extremity of male, lateral view. × 55.

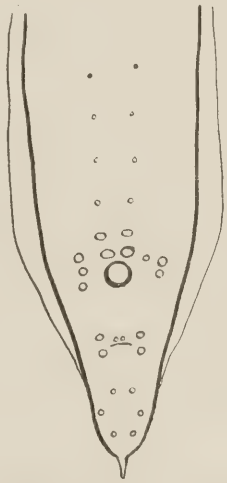

FIG. 15. *Africana africana.* Caudal extremity of male, ventral view. × 80.

conical and ventrally curved ; it measures 0·21 to 0·28 mm. from the cloaca to the extremity which carries a small spike. The caudal papillae number nineteen pairs, twelve preanal, four postanal and three dorsal ; of the preanal there is a subventral line of six in front

of the preanal sucker ; these increase in size from before back, three other pairs are placed subventrally at the side of the sucker, one pair almost laterally, mid-way between sucker and cloaca,* one pair subventrally immediately in front of the cloaca, and one very small pair ventrally on the prominence just in front of the cloaca. Of the postanal papillae three pairs form a ring on the ventral and subventral surface, while the fourth pair is placed subventrally just behind the anus. The three dorsal papillae are posterior to the anus. The preanal sucker is circular in outline, 55 to 66$\mu$ in diameter and has a well-fortified chitinous rim.

The female measures 3·15 mm. to 5·77 mm. in length and 0·21 to 0·375 mm. in maximum diameter ; the vulva, which is not salient, is situated anterior to the middle of the body, 1·8 to 3·67 mm. from the posterior extremity ; the vagina follows a winding course, but in general runs back from the vulva; the ovaries are situated in the anterior part of the body. The caudal extremity is 0·22 to 0·34 mm. in length and tapers off to a very fine point. The eggs have a thick smooth shell and measure 66 by 42$\mu$ to 86 by 46$\mu$ ; they are not segmented when laid.

Table No. II gives detailed measurements from the three known species of this genus, *A. africana*, Gendre (1909), *A. acuticeps* and *A. brodeni*, Gedoelst (1916) with measurements from the worm above described. These species seem to be very closely allied and the only difference of any diagnostic value seems to be the number and arrangement of papillae on the caudal extremity of the male.

* By an oversight I have omitted this papilla in making the final drawing for fig. 15.

TABLE II.

Details of measurements of the species of *Africana*.

| Name ... ... ... | *A. africana* (Gendre, 1909) | | *A. africana* (Gendre, 1909) measurements made from worm described in text | | *A. acuticeps* (Gedoelst, 1916) | | *A. brodeni* (Gedoelst, 1916) | |
|---|---|---|---|---|---|---|---|---|
| Length ... ... | ♂ 5 – 7 | ♀ 6.7 – 8 | ♂ 4.5 – 4.95 | ♀ 3.15–5.77 | ♂ 6.5 – 7 | ♀ 6.8 – 8.5 | ♂ 6.5 | ♀ 7.8 |
| Breadth ... ... | 0.36–0.46 | 0.42–0.48 | 0.321–0.37 | 0.21–0.375 | 0.385–0.39 | 0.4–0.415 | 0.45–0.465 | 0.385 |
| Depth of head ... | ... | ... | 23–29$\mu$ | 26–29$\mu$ | ... | 20$\mu$ | ... | 20$\mu$ |
| Diameter of head ... | ... | ... | 50$\mu$ | 50–68$\mu$ | ... | 47–52$\mu$ | ... | 56$\mu$ |
| Length of pharynx ... | ... | ... | 46–50$\mu$ | 48–60$\mu$ | 65–70$\mu$ | ... | 72$\mu$ | ... |
| Length of oesophagus | ... | ... | 1.12–1.14 | 1.05–1.20 | ... | 1.00 | ... | 1.28 |
| Ratio to entire worm | $\frac{1}{3.8}$ – $\frac{1}{4}$ | $\frac{1}{4}$ – $\frac{1}{4.4}$ | $\frac{1}{4}$ – $\frac{1}{4.3}$ | $\frac{1}{3}$ – $\frac{1}{4.8}$ | $\frac{1}{5}$ | $\frac{1}{5.5}$ | ... | $\frac{1}{4.1}$ |
| Length of bulb ... | ... | ... | ... | 200$\mu$ | ... | 200–230$\mu$ | ... | ... |
| Width of bulb ... | ... | ... | ... | 180$\mu$ | ... | 170–180$\mu$ | ... | 185$\mu$ |
| Length of tail ... | ... | ... | 0.21–0.28 | 0.22–0.34 | 0.24–0.25 | 0.30–0.34 | 0.29 | 0.36 |
| Ratio to entire worm | $\frac{1}{19.5}$ – $\frac{1}{23}$ | $\frac{1}{17}$ – $\frac{1}{17.5}$ | $\frac{1}{21}$ – $\frac{1}{18}$ | $\frac{1}{14.5}$ – $\frac{1}{17}$ | $\frac{1}{21}$ – $\frac{1}{28}$ | $\frac{1}{22}$ – $\frac{1}{25}$ | $\frac{1}{21}$ | $\frac{1}{21}$ |
| Length of spicules ... | 1+ | ... | 1.55–1.73 | ... | 1.8 and 2.0 | ... | 1.85 and 1.69 | ... |
| Ratio to entire worm | $\frac{1}{5}$ | ... | $\frac{1}{2.9}$ – $\frac{1}{2.8}$ | ... | $\frac{1}{3.5}$ | ... | $\frac{1}{3.5}$ | ... |
| Diameter of sucker ... | ... | ... | 63–66$\mu$ | ... | 64$\mu$ | ... | 55$\mu$ | ... |
| Distance of vulva from posterior end | ... | ... | ... | 1.8–3.67 | ... | ... | ... | ... |
| Ratio of above to body length | ... | $\frac{10}{16.6}$ | ... | $\frac{10}{17}$ – $\frac{10}{16}$ | ... | $\frac{10}{17}$ | ... | $\frac{10}{14}$ |
| Eggs, length ... ... | ... | 60–66$\mu$ | ... | 66–86$\mu$ | ... | 64–68$\mu$ | ... | 75–80$\mu$ |
| Eggs, breadth ... | ... | 39–41$\mu$ | ... | 44–46$\mu$ | ... | 40–44$\mu$ | ... | 48–52$\mu$ |

All the above figures are in mm. unless otherwise stated.

### *OXYURIS PRAEPUTIALIS* Skrjabin, 1914

Host :—*Bufo regularis*. Locality :—Nigeria.

Unfortunately only female specimens were available, and as the male has not yet been described, the species cannot be referred to its proper genus. The characters presented by the female are as follows :—

Body white, 2·85 to 4·95 mm. in length by 0·21 to 0·435 mm. in width at its broadest part ; it tapers from the level of the oesophageal bulb towards the head and is usually coiled, or twisted into the form of the letter 'S.' The cuticle is crossed with very fine striations and bears a few minute papillae in the cervical region. The posterior extremity (fig. 16) tapers rapidly behind the anus, and terminates in

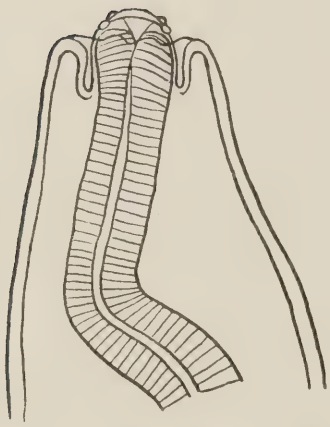

Fig. 16. *Oxyuris praeputialis*. Anterior extremity, head retracted. × 253.

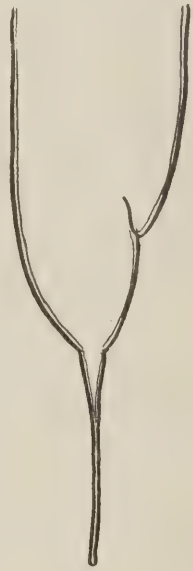

Fig. 17. *Oxyuris praeputialis*. Posterior extremity of female. × 73.

a very fine, straight spike, from 0·24 to 0·495 mm. in length. Small lateral flanges are present, commencing about 0·06 mm. behind the head and terminating about the same distance from the spike at the caudal extremity. The head (fig. 17) is formed by three lips 0·06 to 0·083 mm. wide, each bearing two comparatively large papillae and a short, anterior, marginal membrane. The whole head seems to

be capable of withdrawal within a cuticular fold of the neck and in some specimens it is almost completely hidden there. The nerve ring is situated about 0·16 mm. from the anterior extremity and the excretory pore 0·3 to 0·36 mm. from the same point.

In specimens where the head is retracted, the first portion of the oesophagus is always bent in the form of a double curve ; its diameter is 0·053 to 0·06 mm. and the muscular portion seems to run well up into the bases of the lips. The oesophageal bulb is a well-formed structure, more or less spherical in shape and measures about 0·13 mm. in diameter ; the complete length of the oesophagus is 0·42 to 0·60 mm. The vulva is placed anterior to the middle of the body, 1·85 to 2·7 mm. from the posterior extremity, tracing the vagina inwards it is found, after a short forward bend to turn backward, in which direction it continues to a point near the anus ; the ovaries are situated in the anterior part of the body.

The worm is ovoviviparous ; many specimens were found to contain numerous larvae.

### *TRICURIS DISCOLOR* (Linstow, 1909)

Host :—Cow. Position :—Intestine. Locality :—Lancashire.

Body white, cuticle ringed with striations at intervals of about 7·5$\mu$, contour of the narrow anterior part serrate, two lateral vesicular swellings may be seen at the head.

The male measures 50·25 to 58·75 mm. in length ; the narrow oesophageal portion is 33·75 to 38·5 mm. long and 0·175 mm. broad, and represents about two-thirds of the complete length of the worm ; the thicker posterior portion is 16·5 to 20·25 mm. long and 0·55 to 0·75 mm. at its widest part. The spicule measures 1·95 to 2·3 mm. in length and only 10·0 to 11·5$\mu$ in thickness, being less than half the size of that of *T. ovis* in both measurements (fig. 18). The spicule sheath has a diameter of 165$\mu$ and is covered with spines, these being larger in size and not so closely spaced as in *T. ovis*. Although no specimens were found with spicule extruded, nothing was visible to correspond with the expanded portion at the extremity of the evaginated spicule sheath of *T. ovis*.

The female measures 43·00 to 51·75 mm. in length, the anterior portion is 33·00 to 39·00 mm. long and 0·165 mm. broad and

represents three-quarters of the entire length ; the thicker posterior portion is 10·00 to 12·75 mm. long and 0·825 mm. broad. The egg measures 60 by 30$\mu$ to 73 by 33$\mu$, and, excluding the opercula, 43 to 46$\mu$.

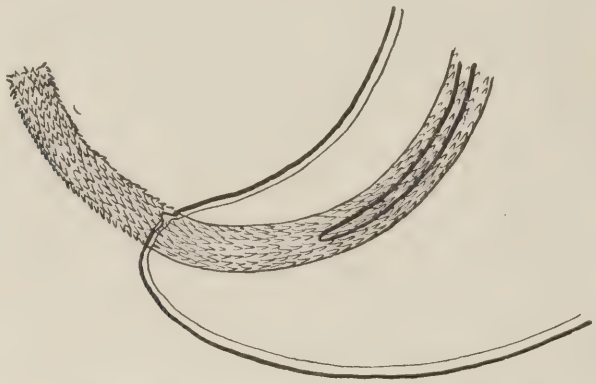

FIG. 18. *Tricuris discolor*. Caudal extremity of male, lateral view. × 190.

So far as can be ascertained, this species has not previously been recorded in this country.

### *PHYSALOPTERA PRAEPUTIALIS* v. Linstow, 1889

Synonym :—*Chlamydonema felineus* Hegt, 1910

Material : four female specimens from the dog corresponding in every way with the description of this species.

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# ON THE HIBERNATION OF ADULT MOSQUITOS

BY

W. REES WRIGHT, B.Sc.

(*Received for publication* 10 *November*, 1924)

The investigations of which the results form the subject of this paper were carried out during the years 1920-24 in a district of North Carnarvonshire lying between the Menai Straits and the Snowdonian mountains. The purpose of the investigations was to endeavour to elucidate the habits of hibernating mosquitos and the relation of certain species of mosquito to man and to his domestic stock.

The field work involved consisted of visiting farm buildings of all types and collecting, for identification and also for use in other studies, the mosquitos seen. The insects were collected in 6-in. by 1-in. specimen tubes; the tube was brought over a resting mosquito, which on taking flight generally flew up into the tube, when it could be secured by means of a wad of cotton wool. Owing to the poor lighting of most of the buildings, the work had to be done in most cases by the light of an electric torch. For a short time a glass tube with gauze covering one end, the other being closed with a rubber 'valve' (as devised by Adie, 1911) was used; it was found that under the cool conditions in which the collecting often was carried out, moisture from the breath condensed on the inner wall of the tube in quantity sufficient to imperil the safety of the insects. It should be remarked that this type of tube has proved of great use in the laboratory as a 'cage,' since the 'valve' enables one to release mosquitos into the tube, or to remove them from it, much more easily than is the case with a tube closed with gauze at both ends.

In the investigation of such a matter as the hibernation of animals, there are two sets of factors which must especially be considered, viz., climatological factors, and environmental factors. Taking the climatological factors first, it may be said that North Carnarvonshire has a climate which is mild but somewhat wet.

The difference between the isotherm for the months of January and July is about 10° C., the former being approximately 5·5° C., and the latter a trifle over 15° C. Away from the Snowdonian mountains a severe frost or a fall of snow is uncommon, while light frosts—sufficient to freeze up road puddles—are not often experienced. It is probable that the relatively slight range of temperature explains certain differences between the results set forth here and those obtained by Sella (1920) and Grassi (1921a and 1923) among others. A temperature of 5° C. is probably less rigorous for a race of animals accustomed to a temperature of only 15° C. than for a race accustomed to something in the neighbourhood of 25° C.

The second group of factors largely resolves itself, in the present instance, into the conditions of the farm buildings and of the animals found therein. The main agricultural pursuit of the area is the grazing of sheep ; while dairy farming is carried out on a small scale, there are only two or three large dairy-farms in the district examined. On a typical farm we find up to a dozen cows, lodged probably in one shed, a few calves in another shed (a ' calf-cot '), two or three horses in their stable, and usually a dozen or more pigs in three or four styes. While practically all farmers keep fowls, it is only a few that do so on any considerable scale. The farm buildings are for the most part old, apart from the dwelling-houses, which in most cases are of far later date—the old dwelling-houses were usually part of the same range of buildings as the animal houses. In consequence of their age, the animal houses are dark, low, ill-ventilated and warm, and often difficult to keep clean. This is more especially the case with the pig-styes and calf-cots and to a lesser degree with the stables. While the other buildings have a flat ceiling—often forming the floor of a loft above—the pig-styes are almost all lean-to in construction ; about thirty inches high in front and six to eight feet high at the back ; very rarely the doorway is placed in the high side, or in the end, but it is even then no larger than will admit a large pig. There is, in consequence, a considerable space overhead in which warm air can accumulate. Except the pig-styes, which are hardly touched, the buildings are whitewashed once a year on most farms, when they get a thorough cleaning ; this usually takes place in early summer. Throughout the winter the stables and cowsheds are cleaned daily ; the other buildings are attended to less frequently or not at all.

The pig-styes generally have masses of cobwebs dependent from their ceilings.

Throughout Northern Europe only three species of mosquito are known to hibernate as the adult, viz., *Anopheles maculipennis*, *Theobaldia annulata* and *Culex pipiens*. In the autumn of 1920, when I commenced my investigations, our knowledge of the phenomena of hibernation was not far advanced from the point where Annett and Dutton left it in 1901. Thus Hindle (1914) briefly states that

> 'In cold regions mosquitos pass through the winter in the egg-stage, but in addition some of the females hibernate in dark corners.'

Patton and Cragg (1913) state that

> 'Mosquitos, like all other insects which normally have a short life-history and which can only multiply under certain conditions are able to exist . . . . during unfavourable seasons. . . . . The commonest manner in which this is brought about is by the hibernation of impregnated females, which, finding the season too advanced to complete the maturation and deposition of their ova, seek out resting places and remain concealed until favourable conditions present themselves at the commencement of the next season. During this period, which may extend to several months in temperate climates such as those of Europe, they feed seldom if at all, and remain in a passive and torpid condition, living upon the store of food material already accumulated, until they are revived by the warm weather.'

Finally, Lang (1918) only cites Annett and Dutton's work on hibernation.

Since the autumn of 1920, a number of excellent papers on the hibernation of mosquitos have appeared; to mention but a few, those of Grassi and of Sella from Italy (*op. cit. supra*), of Osterwald and Tänzer (1920) from Saxony, and probably most important of all, Wesenburg-Lund's memoir on the Danish mosquitos. There have been close approximations between the results obtained by the more Northern workers—*e.g.* Wesenburg-Lund (1921), Osterwald and Tänzer, and myself, and between the results of more Southern workers such as Sella and Grassi; as I have suggested above, a few variations, which will be pointed out in due course, are probably climatic in origin.

The three species of mosquito which have already been mentioned are found in North Carnarvonshire. In the summer the larvae are to be found in almost every ditch, *Anopheles* being particularly abundant. During the winter neither of the Culicine species are as abundant as one would expect to find them. In consequence of this,

and also because of its far greater importance from the point of view of medicine, most attention has been paid to *Anopheles*.

Roubaud has recently (1923) proposed a physiological basis for hibernation. He suggests that the hibernation is brought about, not by the increasing cold, but by an auto-intoxication of the insects by a 'renal surcharge.' He believes that the latest-hatched members of the summer generations inherit a 'patrimoine d'intoxication' which gives rise to the peculiar physiological conditions of the hibernating female. Among others, this condition manifests itself physically by a hypertrophy of the malpighian tubules, physiologically by the fact that any blood ingested goes to form fat body, instead of assisting in the maturation of the ova. It is only when this intoxication has worked itself off, which does not happen till early spring, that the eggs develop. As far as North Wales is concerned, I have not observed either the physical or physiological signs of this intoxication; out of numbers of mosquitos dissected during the winter months, in none did I observe any abnormal state of the malpighian tubules, while I discovered that both in the laboratory and also under natural conditions, female mosquitos matured their eggs as early as January.

Grassi (1921a) and also Sella (1920) have drawn a distinction between true or rigorous hibernation and partial hibernation. The former is defined as a condition in which the insect develops fat-body towards the late autumn, and does not then feed until spring, while in the latter stage it continues to feed and does not become fat. Grassi believes that it is the former class of females that perpetuates the species, and that the latter die off before the spring. According to Roubaud (1923) this is also the case in France. In the former state the insect does not move about. Whatever may be the conditions on the European continent, where the mosquitos are subjected to a greater variation of temperature than occurs in this country, in Carnarvonshire I found *Anopheles* flying actively within the farm buildings throughout the winter, save when the temperature fell to somewhere near zero Centigrade. The insect is willing to feed at all times except immediately after a blood meal. Yorke and Macfie, experimenting at the Liverpool School of Tropical Medicine with *Anopheles* which I had sent them from North Wales, found that a considerable number died in their incubators, apparently as the

result of the development of their eggs, which they could not deposit owing to lack of facilities. I have observed the development of eggs in wild *Theobaldia* as early as January.

*Anopheles maculipennis.* This species is, throughout the year, an inhabitant of farm buildings, as Roubaud (1920) and Wesenburg-Lund (1921), among others, have pointed out. During the winter females alone occur.

It occurs chiefly in the pig-styes ; next, in order of frequency, in calf-cots, cowsheds and stables—very rarely in these last. In lofts and barns it rarely occurs—never, I think, unless these are in communication with a chamber containing animals. I have only once found it in a fowl-house. Taking at random from my notes a series of twelve farms examined during the latter part of November and the first part of December, 1923, I find that I recorded it as occurring abundantly in six groups of pig-styes, and as being less abundant in four other groups. It was once recorded as abundant in cowsheds, and four times as occurring in these buildings. It was once recorded from a stable, and twice from lofts or barns. At one farm where it was absent from the pig-styes, these had been empty for at least two months, while at one farm there were no buildings other than cowsheds and their lofts. It occurred in pig-styes about five times ; if a longer series of records were to be taken, the attraction which pig-styes have for mosquitos would be even more marked.

In whatever buildings *Anopheles maculipennis* occurs, it is more abundant towards the upper parts, as was pointed out by Annett and Dutton (1901). Frequently it rests on cobwebs. As a general rule, it will be found in the more dimly-lit places, such as the sides of beams furthest from the light. As has already been stated, it feeds throughout the winter ; and at least 50 per cent. of the mosquitos collected during these investigations contained recognisable blood.

Usually the insects take up the resting-position so often described, but, as Annett and Dutton pointed out, under certain conditions, especially cold, they take up a *Culex*-like attitude. Under the influence of cold they become exceedingly torpid and when touched make no effort to fly. They rapidly recover in a warmer place, however. Normally the insects are active, flying spontaneously

both in captivity and in nature. A bright light, as well as the approach of some object, causes them to take flight; in this my experience has been different to that of Roubaud (1918) who found that a bright light caused flying mosquitos to come to rest.

The attraction which the animals' quarters have for this mosquito is probably due to several causes. I am of opinion that the insect's main desiderata are darkness, warmth, and easily-accessible food. This last condition will be fulfilled by dwelling-houses as well as animal houses, and need not be taken into consideration. In the area with which I am acquainted, it is the pig-styes which fulfil the first two conditions most completely, while the animal houses as a whole are darker, warmer and quieter than the dwelling-houses. It may be that an attraction towards the animal houses arising from these causes may be in progress of giving rise to a zoophile race of mosquitos, as has been supposed by Roubaud (1920) and by Grassi (1921b). It is possible that the comparative hairlessness of the pig, and the fact that its skin, though thick, is highly vascular—affording a readily-tapped source of blood—may be additional attractions for the insect.

The Malaria Committee for the Province of North Holland (1920) found that mosquitos were most numerous in pig-styes. On the other hand Vogel (1921), working in the zone occupied by the Fifth German Army during the late War, found that cowsheds were preferred to pig-styes.

*Theobaldia annulata.* This species also hibernates in the adult stage, the females alone surviving the winter. Like *Anopheles* it is an inhabitant of the animal quarters, but occurs also in the colder buildings, such as store-houses, etc., and occasionally in dwelling-houses. I think, however, that it resorts to these last only when no other buildings where food may be obtained are available. Like *Anopheles*, it feeds throughout the winter. It rests on cobwebs as a rule, on the lower part of a sloping ceiling or on the wall, and takes up the hunchback *Culex* attitude.

On 10th January, 1924, I obtained, in some cellars at the University College of North Wales, Bangor, two females of this species which contained matured eggs. They were given a blood meal, and placed in a breeding cage, where both oviposited the same

day ; the temperature of the laboratory was about 13° Centigrade. The larvae hatched out on the 21st, the maximum temperature in the interim being 14·7° C. and the minimum 6·7° C.—mean temperature about 13° C. Unfortunately, the larvae were destroyed through an accident to the breeding tank.

In 1924, both this species and *Anopheles* left the farm buildings towards the end of March, but larvae were not found till mid-May.

*Culex pipiens.* Unlike the two previously-mentioned species, *Culex* hibernates almost exclusively in cellars, lofts and similar cool places. It is a more lethargic insect than the other two, spending most of its time resting on the walls and on cobwebs ; it is found towards the lower part of the walls. When disturbed it flies actively. This species is supposed to be more partial to avian blood than to mammalian. I have only rarely taken specimens containing blood, and, unfortunately, on those occasions I have had no facilities for determining the nature of the blood, whether mammalian or avian. In the laboratory this species will not feed on man in my experience.

During 1923-24 my attention was directed, in addition to the main investigation, towards the possibility of reducing the number of *Anopheles* in a district, by the collection or destruction of the hibernating adults. One farm was particularly well-adapted for investigations of this nature, since the breeding-place of the mosquitos found in it was known to be a small pond actually within the farmyard, and there were no other places near-by—either hibernating quarters or breeding-places—from which an influx of mosquitos might be expected. All mosquitos seen were collected—many hundred *Anopheles* and a few *Culex* and *Theobaldia*. In the course of this work it was found that if a building, *e.g.*, a pig-stye, was apparently cleared of mosquitos one day, more would be found there the next. Having regard to the construction of the buildings, I am inclined to interpret this as being due to emergence of mosquitos from recesses in which they could not be detected, rather than to an actual migration ; a certain amount of migration between adjoining buildings undoubtedly takes place, when there are any direct communications such as holes in the walls. Buildings were apparently cleared by several collections.

Despite the collecting, *Anopheles*, *Theobaldia* and *Culex* appeared

in these breeding-places in early summer, and were abundant in the buildings, and I am forced to the conclusion that the collection of hibernating mosquitos is by itself of no value as a means of control

## SUMMARY

1. *Anopheles maculipennis, Theobaldia annulata* and *Culex pipiens* hibernate as adult females.

2. *Anopheles maculipennis* occurs in those farm buildings which contain animals, especially in pig-styes ; it occurs also in calf-cots, cowsheds and stables, and, very occasionally, in lofts or barns. When these communicate with a building containing animals, the anopheline flies spontaneously at any time of day. It feeds throughout the winter. Under laboratory conditions, the females will develop eggs during the winter, if kept at a sufficiently high temperature.

3. *Theobaldia annulata* occurs in the same type of building as does *Anopheles* and also in cooler places such as lofts and cellars. Like *Anopheles*, it feeds throughout the winter and flies spontaneously. Females which contain matured eggs have been caught in January.

4. *Culex pipiens* occurs in cool buildings and cellars exclusively. It is much less active than the other two species.

5. Experiments to determine the effect of collecting the hibernating females have shown that this alone is not a useful means of control.

## ACKNOWLEDGMENTS

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School of Tropical Medicine, for much helpful criticism and for making it possible for me to have access to the Library of the School ; and to H.M. Department of Scientific and Industrial Research for a research grant.

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# MISCELLANEA

## CORRIGENDA

" Descriptions of new mosquitos from South America "
(*Ann. Trop. Med. & Parasit.*, Vol. XVIII, No. 3, pp. 363-375).

*Culex* (*Choeroporpa*) *innominatus*, sp.n. Evans (*loc. cit.*, p. 365).
For ' paratypes ' and ' cotypes ' respectively, read :
' Cotypes : 2♂♂ River Amazon, 1915 (Dr. Aiken Clark) ; 2♂♂ Palo Negro, Venezuela, 30.8.22, and 1♂ Mariara, Estada Carabobo, Venezuela, 11.8.22 (Dr. M. Núñez Tovar).'

*Culex* (*Choeroporpa*) *clarki*, sp.n. Evans (*loc. cit.*, p. 367).
For ' paratypes ' read ' cotypes.'

*Culex innovator*, sp.n. Evans (*loc. cit.*, p. 375).
For ' paratypes ' read ' cotypes.'

ALWEN M. EVANS,
10*th November*, 1924.

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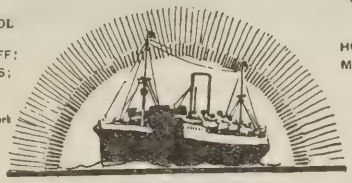

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References to authors in the text must be made in the following way :— 'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way :—

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